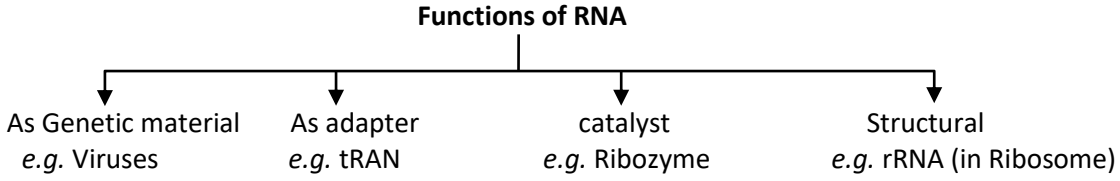
Molecular Basis of Inheritance

INTRODUCTION OF MOLECULAR BIOLOGY

MOLECULAR BASIS OF INHERITANCE

- Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are two types of nucleic acids, found in living systems.

  F. Meischer discovered nucleic acid in nucleus of pus cell and called it "nuclein". The term nucleic acid was coined by **"Altman"**.
- DNA acts as the genetic material in most of the organisms. Though RNA also acts as a genetic material in some viruses but mostly functions as a messenger. RNA has additional roles as well, It functions as adapter, structural, and in some cases as a catalytic molecule. Nucleic acids are polymers of nucleotides.



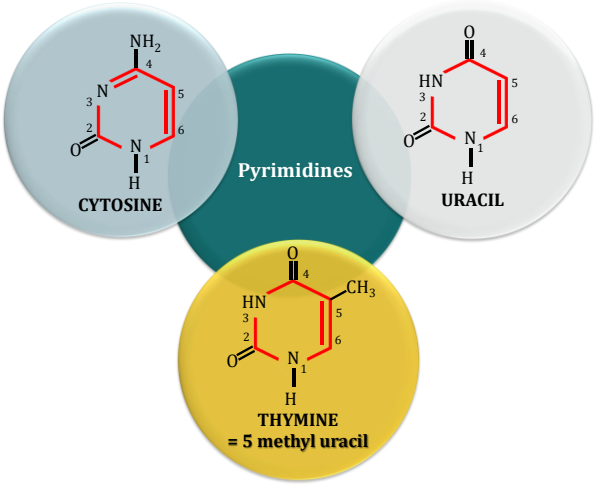
NUCLEOTIDE : Nucleotides are known as monomers of Nucleic acid. A nucleotide has three components.

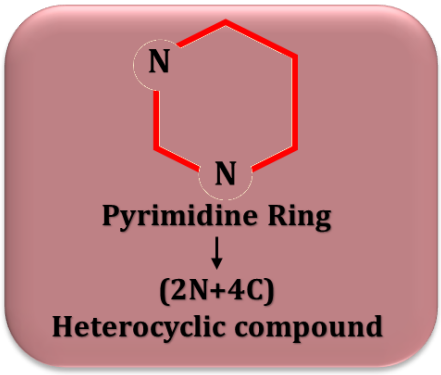
1. Nitrogen base
2. Pentose sugar
3. Phosphate

A. NITROGEN BASE :

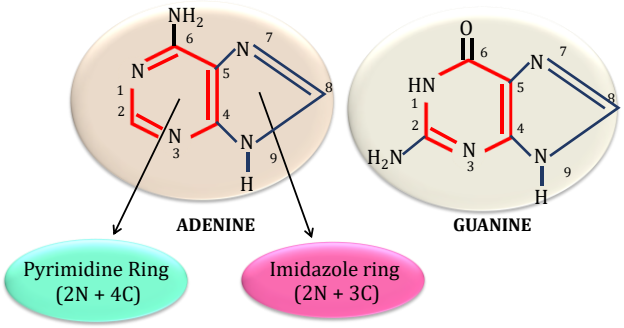
On the basis of structure nitrogen bases are of two types :

(1) **Pyrimidines –** Consist of one pyrimidine ring. Skeleton of ring composed of two nitrogen and four carbon atoms. **e.g.** Cytosine, Thymine and Uracil.



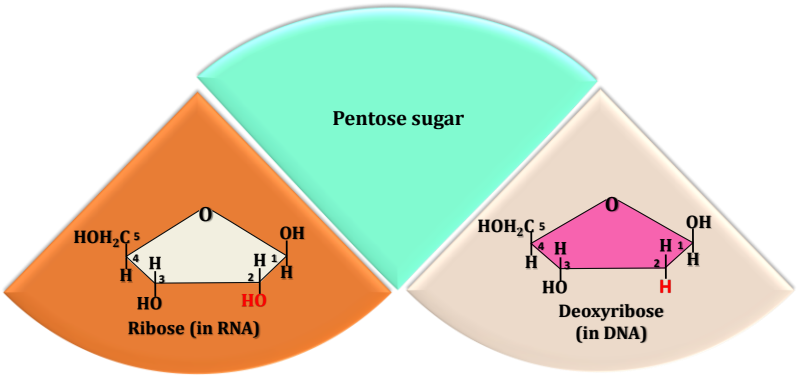


(2) **Purines –** Consist of two rings i.e. one pyrimidine ring (2N + 4C) and one imidazole ring (2N + 3C) **e.g.** Adenine and Guanine.



B. PENTOSE SUGAR :

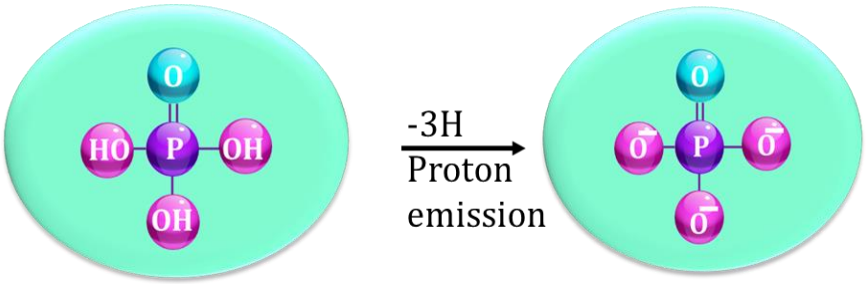
Number of carbon atoms is five. DNA has Deoxy ribose sugar. RNA has ribose sugar



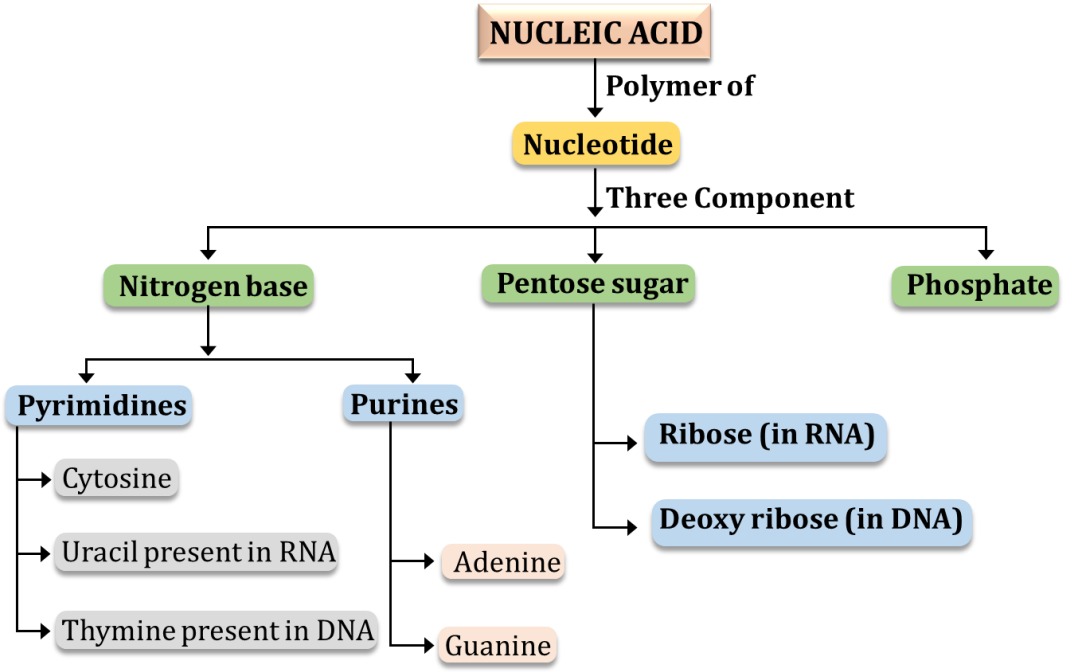
C. PHOSPHOTE :

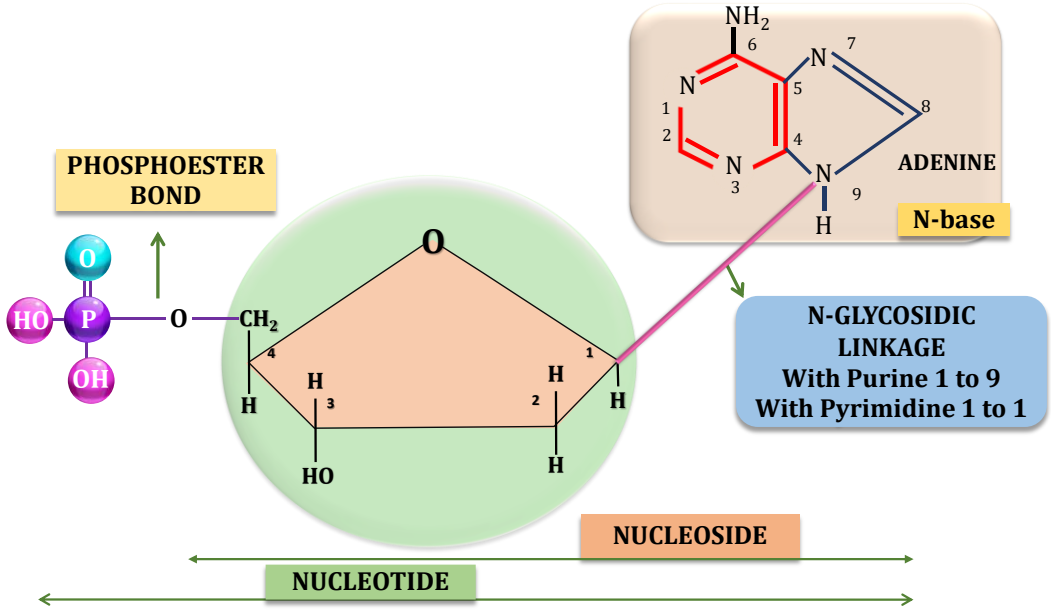
These are acidic and negatively charged.

Phosphate forms phosphodiester bond (covalent bond) with fifth Carbon of sugar to form a complete nucleotide.



Nitrogen base forms bond with first carbon of pentose sugar to form a nucleoside. Nitrogen of first place (N_1) forms bond with sugar in case of pyrimidines while in purines nitrogen of ninth place (N_9) forms bond with sugar.





NAMING OF NUCLEOTIDES : For RNA

N-base	Nucleosides = N base + Ribose sugar	Nucleotides = Nucleoside + P
Adenine	Adenosine	Adenylic acid (AMP)
Guanine	Guanosine	Guanylic acid (GMP)
Cytosine	Cytidine	Cytidylic acid (CMP)
Uracil	Uridine	Uridylic acid (UMP)

NAMING OF NUCLEOTIDES : For DNA

N-base	Nucleosides = N base + Deoxy Ribose sugar	Nucleotides
Adenine	Deoxyadenosine	Deoxyadenylic acid ($_d$AMP)
Guanine	Deoxyguanosine	Deoxyguanylic acid ($_d$GMP)
Cytosine	Deoxycytidine	Deoxycytidylic acid ($_d$CMP)
Thymine	Deoxythymidine	Deoxythymidylic acid ($_d$TMP)

NCERT Insider :-

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two types of nucleic acids found in living systems. DNA acts as the genetic material in most of the organisms. RNA though it also acts as a genetic material in some viruses.

Question-1 Which of the following is a nucleoside in RNA?

(1) Deoxy guanosine (2) Cytosine (3) Uridylic acid (4) Adenosine

Answer: (4) Adenosine

Question-2: Nucleotide consist of ?

(1) N_2 - base, pentose sugar and phosphoric acid
(2) Nitrogen, Hexoses sugar and phosphoric acid
(3) Nitrogen base, pentose sugar
(4) Nitrogen base, trioses and phosphoric acid

Answer: (1) N_2 - base, pentose sugar and phosphoric acid

Explanation:

A Nucleotide have three component
e.g. 1-Nitrogen base, 2-pentose sugar, 3-phosphate (Phosphoric acid).

Question-3: Which of following sugar present in DNA?

(1) Phosphate (2) Ribose sugar (3) Deoxy ribose (4) Nitrogen base

Answer: (3) Deoxy ribose

Explanation:

The Pentose **sugar present in DNA** is called deoxyribose contains five carbon atoms.

Question-4: Who gave the term nucleic acid :-

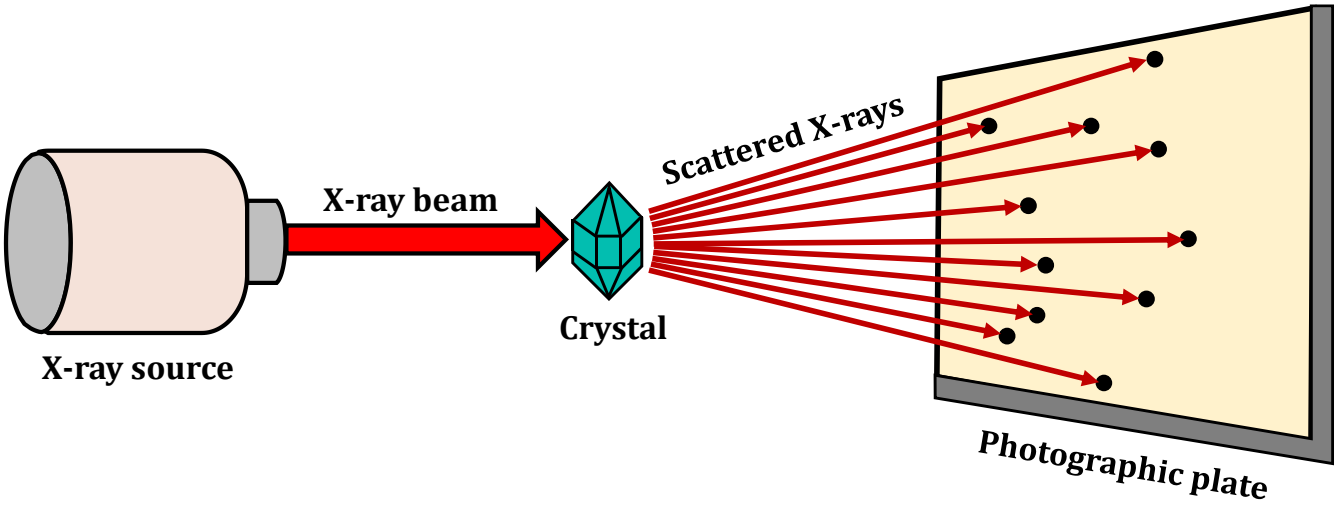
(1) Altman (2) Crick (3) Kornberg (4) Singer and Nicolson

Answer: (1) Altman

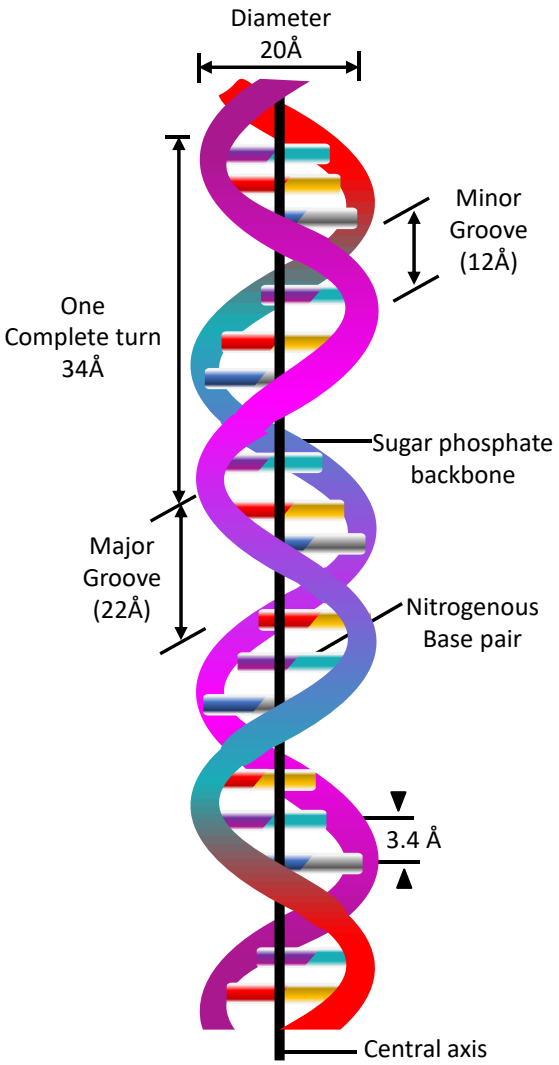
NUCLEIC ACIDS

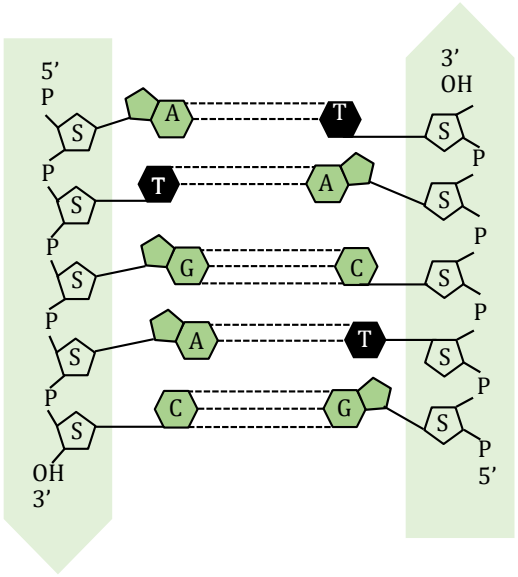
DNA (Deoxyribo Nucleic Acid) :

* DNA as an acidic substance, present in nucleus, was first identified by Friedrich Meischer in 1869.
* DNA term was given by – Zacharias
* DNA is long polymer of deoxyribonucleotides.
* DNA is negatively charged (due to presence of Phosphate)
* In DNA pentose sugar is deoxyribose sugar and four types of nitrogen bases A,T,G,C
* Wilkins and Franklin studied DNA molecule with the help of X-ray crystallography .



* Watson and Crick (1953) proposed a double helix model for DNA. For this model Watson, Crick and Wilkins were awarded by Noble Prize in 1962.
* One hallmark (main point) of double helix model is complementary base pairing between purine and pyrimidine.
* According to this model, DNA is composed of two polynucleotide chains.
* Both polynucleotide chains are complementary and antiparallel to each other.
* **In both strand of DNA direction of phosphodiester bond is opposite. i.e. If direction of phosphodiester bond in one strand is 3'-5' then it is 5'-3' in another strand.**
* Both strands of DNA are held together by hydrogen bonds. These hydrogen bonds are present between nitrogen bases of both strand.
* Adenine binds to thymine by two hydrogen bonds and cytosine binds to guanine by three hydrogen bonds.
* **In a DNA molecule one purine always pairs with a pyrimidine. This generates approximately uniform distance between the two strands of DNA.**
* **In DNA plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, confers stability of the helical structure of DNA.**





NCERT Insider :-

The two chains are coiled in a right-handed fashion. The pitch of the helix is 3.4 nm (a nanometre is one billionth of a metre, that is 10-9 m) and there are roughly 10 bp in each turn. Consequently, the distance between a bp in a helix is approximately 0.34 nm.

Question-1: DNA term was given by :-

(1) Zacharias
(2) Schleiden and Schwann
(3) Singer and Nicolson
(4) Kornberg and Khurana

Answer: (1) Zacharias

Question-2: Who studied DNA molecule with the help of X-Ray crystallography ?

(1) Wilkins and Franklin
(2) Schleiden and Schwann
(3) Singer and Nicolson
(4) Kornberg and Khurana

Answer: (1) Wilkins and Franklin

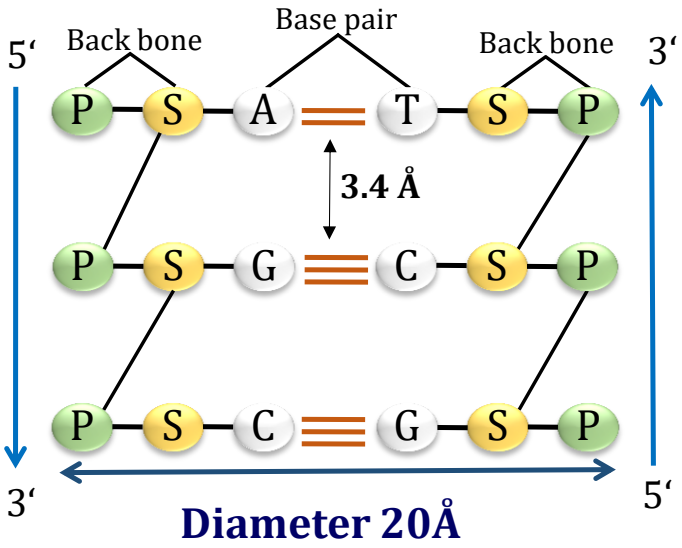
Question-3: Substance common in DNA and RNA -

(1) Hexose Sugar
(2) Uracil
(3) Thymine
(4) Adenine

Answer: (4) Adenine

STRUCTURE AND TYPES OF DNA

* Two strands of DNA are helically coiled like a revolving ladder. Back bone of this ladder (reiling) is composed of phosphates and sugars while steps (bars) are composed of pairs of nitrogen bases.
* Two chains have anti-parallel polarity. It means, if one chain has the polarity 5' $\rightarrow$ 3', the other has 3' $\rightarrow$ 5'.
* Distance between two successive steps is 3.4 Å. In one complete turn of DNA molecule there are such 10 steps (10 pairs of nitrogen bases). So the length of one complete turn is 34 Å. This is called helix length.
* Diameter of DNA molecule i.e. distance between phosphates of two strands is 20 Å.



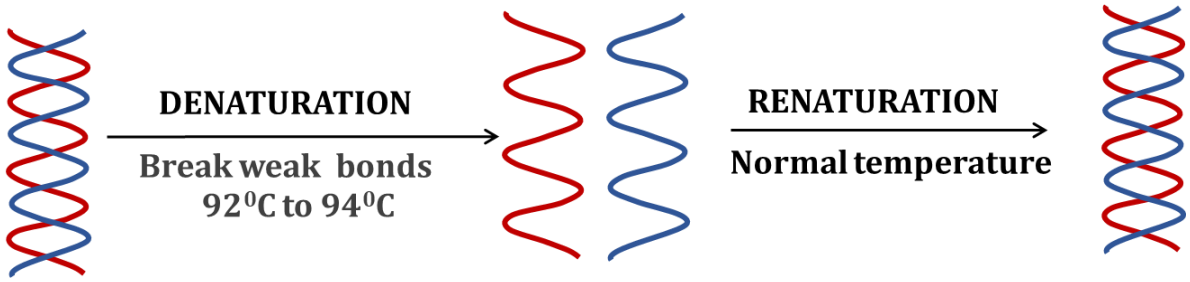
* Each step of ascent is represented by a pair of bases. At each step of ascent, the strands turns 36°.
* In nucleus of eukaryotes the DNA is associated with histone protein to form nucleoprotein.
* Bond between DNA and histone is salt linkage (Mg^{+2}).
* $\phi \times 174$ bacteriophage has a single stranded circular DNA, it was discovered by **Sinsheimer**.
* G-4, S-13, M-13 F and Fd bacteriophages also have single stranded circular DNA.

$\phi \times 174$ (bacteriophage) [Single stranded]	5386 Nucleotides
λ bacteriophage	48502 base pair
E.coli	4.6×10^6 base pair
Human (Haploid cell)	3.3×10^9 base pair
Human (Diploid cell)	6.6×10^9 base pair

DENATURATION AND RENATURATION OF DNA

If a normal DNA molecule is placed at high temperature (80-94°C) then both strands of DNA will separate from each other due to breaking of hydrogen bonds. It is called DNA-denaturation.

When denatured DNA molecule is placed at normal temperature then both strand of DNA attached and recoiled to each other. It is called renaturation of DNA.



Question-1: In DNA molecule distance between two successive steps is :-

(1) 3.4 Å
(2) 34 Å
(3) 20 Å
(4) 3.4 nm

Answer: (1) 3.4 Å

Question-2: Number of base pairs present in haploid cell of human :-

(1) 3.3×10^9 base pair
(2) 4.3×10^9 base pair
(3) 4.6×10^6 base pair
(4) 6.6×10^9 base pair

Answer: (1) 3.3×10^9 base pair

Question-3: In DNA molecules length of one turn of DNA is

(1) 3.4 Å
(2) 34 Å
(3) 20 Å
(4) 16 Å

Answer: (2) 34 Å

Question-4: Double helix model of DNA is proposed by

(1) Watson and Crick
(2) Schleiden and Schwann
(3) Singer and Nicolson
(4) Kornberg and Khurana

Answer: (1) Watson and Crick

Question-5: Back bone structure of DNA molecule is made up of :-

(1) Pentose Sugar and phosphate
(2) Hexose sugar and phosphate
(3) Purine and pyrimidine
(4) Nitrogen base and phosphate

Answer: (1) Pentose Sugar and phosphate

STRUCTURE AND TYPES OF DNA

TYPES OF DNA :-

On the basis of direction of twisting, there are two types of DNA.

RIGHT HANDED DNA :-

The DNA for which Watson and Crick proposed model was 'B' DNA. It was a right handed DNA. It shows Clockwise twisting

DNA	Helix Length	No. of Base pairs	Distance between two pairs	Diameter
'B'	34 Å	10 pairs	3.4 Å	20 Å

LEFT HANDED DNA :-

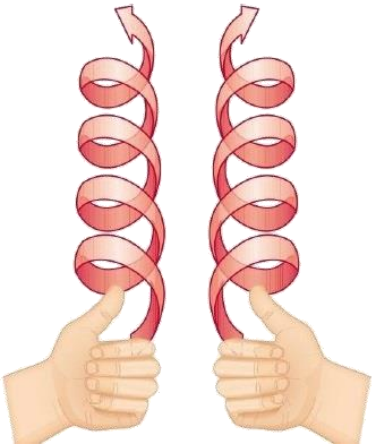
Anticlockwise twisting e.g. Z-DNA - discovered by Rich. Phosphate and sugar backbone is zig-zag.

Helix length – 45.6 Å

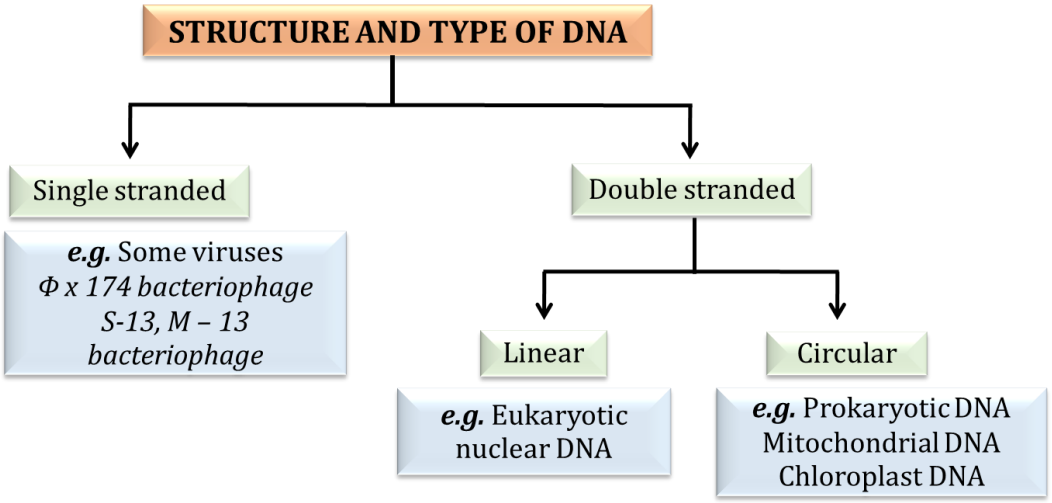
Diameter – 18.4 Å

No. of base pairs – 12 (6 dimers)

Distance between 2 base - pairs – 3.75 Å



Left handed DNA **Right handed DNA**



PALINDROMIC DNA :-

Wilson and Thomas (1974) used this term

Sequence of nucleotides same from both ends.

(In double standard DNA where in reading 5′ to 3′ forward on one strand matches the sequence reading backward 5′ to 3′ on the complementary strand).

CHARGAFF'S EQUIVALENCY RULE –

In a double stranded DNA amount of purine nucleotides is equals to amount of pyrimidine nucleotides.

Purine = Pyrimidine

$$[A] + [G] = [T] + [C]$$

$$\frac{[A]+[G]}{[T]+[C]} = 1$$

So, A = T & G = C

Base ratio = $\frac{A+T}{G+C}$ = constant for a given species. i.e. species specific.

In a DNA A + T > G + C $\Rightarrow$ A – T type DNA. Base ratio of A – T type of DNA is more than one.

e.g. Eukaryotic DNA

In a DNA G + C > A + T $\Rightarrow$ G – C type DNA. Base ratio of G – C type of DNA is less than one.

e.g. Prokaryotic DNA

If G + C > A + T $\rightarrow$ G – C Type/rich DNA $\rightarrow$ High melting point

Melting point of DNA depends on G – C contents.

More G – C contents means higher melting point.

T_m = Temperature of melting.

T_m of prokaryotic DNA > T_m of Eukaryotic DNA

Question-1: Right handed DNA shows ?

(1) Clockwise twisting (2) Anti clockwise twisting

(3) DNA never shows twisting (4) Both (1) and (2)

Answer: (1) Clock wise twisting

Question-2: Which of the following is left handed DNA :-

(1) A DNA (2) B DNA (3) C DNA (4) Z DNA

Answer: (4) Z DNA

Question-3: In a double strand DNA amount of Guanine is 35% then what will be the amount of adenine

(1) 70% (2) 15% (3) 30% (4) 35%

Answer: (2) 15%

Question-4: Consider the following data :-

A = 35%, G = 20%, C = 15%, T = 30%

It may be -

(1) dsDNA (2) dsRNA (3) ssDNA (4) ssRNA

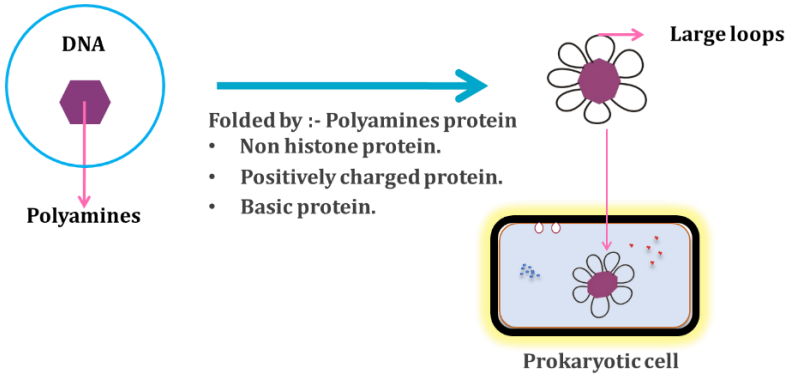
Answer: (3) ssDNA

PACKAGING OF DNA HELIX

- The average distance between the two adjacent base pairs of 0.34 nm (0.34×10^{-9} m or 3.4 Å). Length of DNA for a human diploid cell is 6.6×10^9 bp $\times$ 0.34×10^{-9} m/bp = 2.2 m. The length is far greater than the dimension of a typical nucleus (approximately 10^{-6} m).
- The number of base pairs in ***Escherichia coli*** is 4.6×10^6. The total length is 1.36 mm. The long sized DNA accommodated in small area (about 1 mm in ***Escherichia coli***) only through packing or compaction. DNA is acidic due to presence of large number of phosphate group. Compaction occurs by folding acid attachment of DNA with basic proteins, polyamine in prokaryotes and histone in eukaryotes.

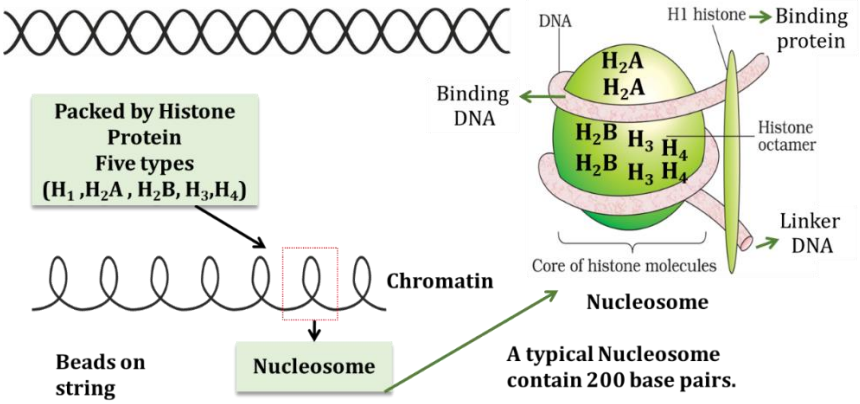
DNA PACKAGING IN PROKARYOTES :

DNA is found in cytoplasm in supercoiled state. The coils are maintained by non-histone basic protein like polyamines. This compact structure of DNA is called nucleoid or genophore.



DNA PACKAGING IN EUKARYOTES :

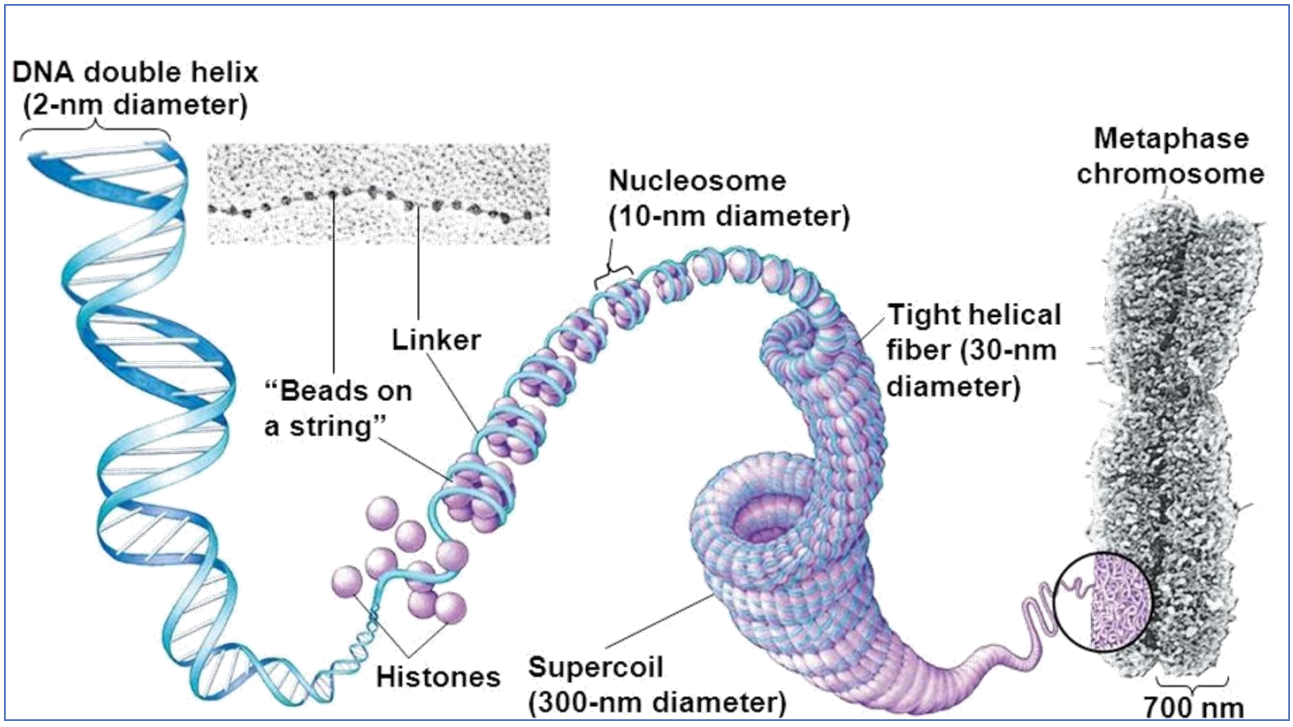
It is carried out with the help of lysine and ariginine rich basic proteins called histone. The unit of compaction is nucleosome. There are five types of histone proteins H_1 H_2A, H_2B, H_3 and H_4. Four of them occur in pairs to produce histone octamer (2 copies of each – H_2A, H_2B, H_3 and H_4), called nubody or core of nucleosome. Their positively charged ends are directed outside.



- The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called nucleosome. A typical nucleosome contains 200 bp of DNA Helix. DNA present between two adjacent nucleosome is called linker DNA. It is attached to H_1 histone protein. Length of linker DNA varies from species to species. Nucleosome chain gives a beads on string appearance under electron microscope. The nucleosomes furthers coils to form solenoid. It has diameter of 30 nm as found in chromatin. The beads on string structure in chromatin is packaged to form chromatin fibres that are further coiled and condensed at metaphase stage of cell division to form chromosomes. The packaging at higher level requires additional set of proteins (acidic) that collectively are referred to as non-histone chromosomal (NHC) proteins.

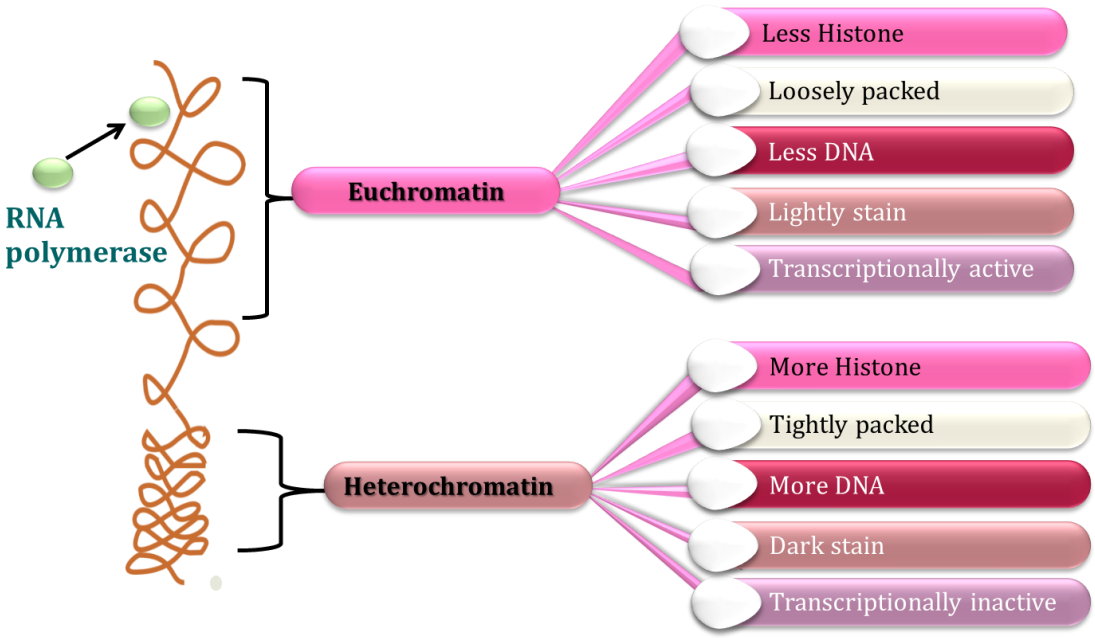
NON-HISTONE CHROMOSOMAL PROTEINS ARE OF THREE TYPES :

(i) Structural NHC protein
(ii) Functional NHC protein e.g., DNA polymerase, RNA polymerase
(iii) Regulatory NHC protein



EUCHROMATIN AND HETEROCHROMATIN :

In a typical nucleus, some region of chromatin are loosely packed (and stains light) and are referred to as euchromatin. The chromatin that is more densely packed and stains dark is called as heterochromatin, specifically euchromatin is said to be transcriptionally active and heterochromatin is transcriptionally inactive.



Question-1: Nucleosome core is consists of?

(1) Eight subunits of proteins (2) Four subunits of proteins
(3) Two subunits of proteins (4) Sixteen subunits of proteins

Answer: (1) Eight subunits of proteins

Question-2: Which of the followings is transcriptionally active?

(1) Telomere (2) Centromere (3) Heterochromatin (4) Euchromatin

Answer: (4) Euchromatin

Question-3: Which of the following is positively charged basic protein ?

(1) DNA (2) Histone (3) RNA (4) Phosphate

Answer: (2) Histone

Explanation:

Histones are rich in basic amino acid (AA) residues such as lysine and arginine. These two amino acids carry a positive charge at their side chains. Due to this reason, histones are called as the set of positively charged basic proteins.

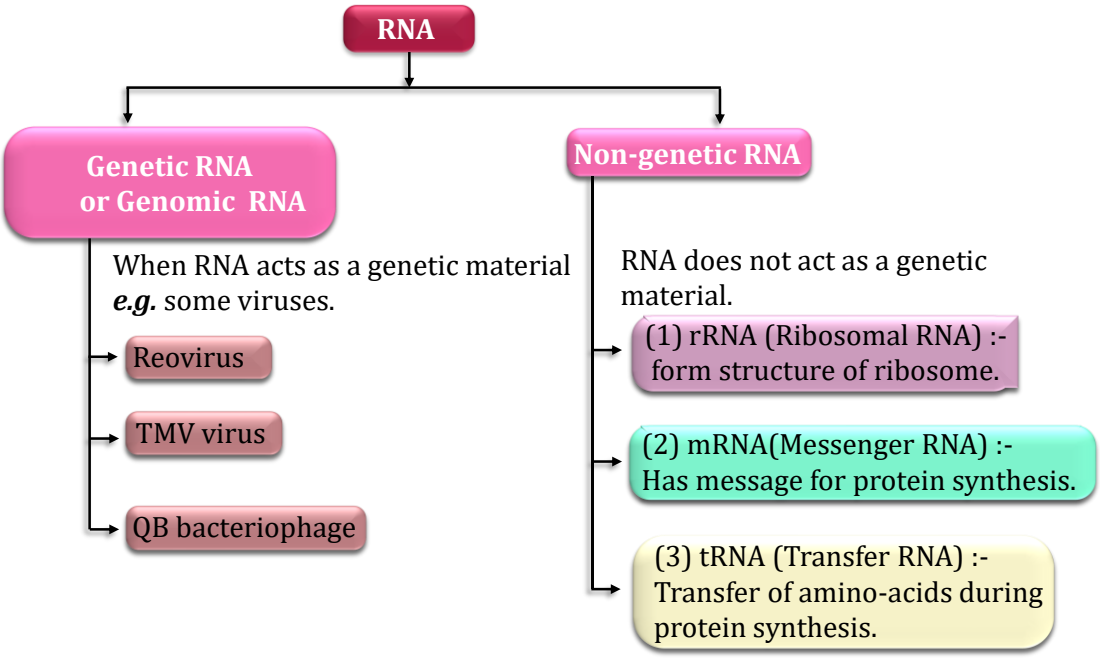
STRUCTURE AND TYPE OF RNA

Structure of RNA is fundamentally same as DNA, but there are some differences. The differences are as follows:-

(1) In place of Deoxyribose sugar in DNA, there is present Ribose sugar in RNA.
(2) In place of nitrogen base Thymine in DNA, there is present uracil in RNA.
(3) RNA is made up of only one polynucleotide chain i.e. RNA is single stranded.
(4) RNA functions as adapter, structural and in some cases as a catalyst (Ribozyme)

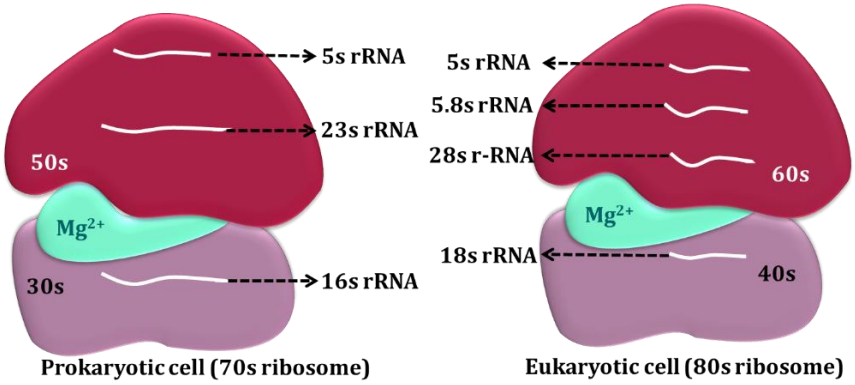
Exception :

RNA found in Reovirus is double stranded, i.e. it has two polynucleotide chains.


RNA
Genetic RNA or Genomic RNA
Non-genetic RNA
When RNA acts as a genetic material *e.g.* some viruses.
Reovirus
TMV virus
QB bacteriophage
RNA does not act as a genetic material.
(1) rRNA (Ribosomal RNA) :- form structure of ribosome.
(2) mRNA(Messenger RNA) :- Has message for protein synthesis.
(3) tRNA (Transfer RNA) :- Transfer of amino-acids during protein synthesis.

(1) RIBOSOMAL RNA (RRNA) :-

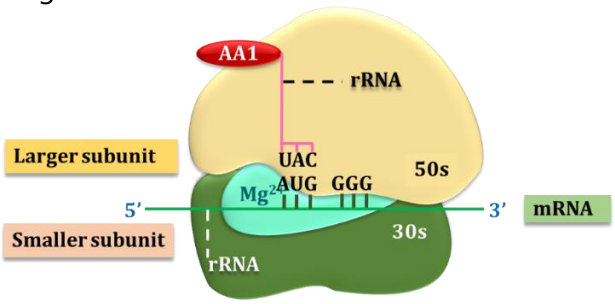
* This RNA is 80% of the cell's total RNA
* rRNA was discovered by Kuntze.
* It is found in ribosomes.
* It is the most stable form of RNA.
* These are present in **80s type** of ribosomes in Eukaryotic cells. Their subunits are **60s** and **40s**. In 60s sub unit of ribosome three types of rRNA are found – 5s, 5.8s, 28s.
* In eukaryotic cell, maximum rRNA are produced in nucleolus except 5S rRNA.
* 5S rRNA is produced in nucleoplasm.
* 40s sub unit of ribosome has only one type of rRNA i.e. 18s.
* So 80s ribosome has total 4 types of rRNA.
* Prokaryotic cells have 70s type of ribosomes and its subunits are 50s and 30s.
* 50s sub unit of ribosome contains 2 types of rRNA i.e. 5s and 23s
* 30s sub unit of ribosome has 16s type of rRNA.
* So 70s ribosome has total 3 types of r–RNA.



Function :

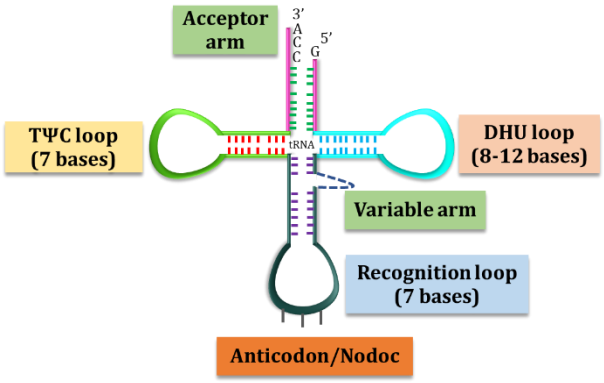
* At the time of protein synthesis, rRNA provides attachment site to tRNA and mRNA and attaches them on the ribosome.

* It attaches tRNA to the larger subunit of ribosome and mRNA to smaller subunit of ribosome.



(2) TRANSFER – RNA (tRNA) :–

* It is 10-15% of total RNA.
* It is synthesized in the nucleus by DNA.
* It is also known as soluble RNA (sRNA)
* It is also known as Adapter RNA.
* It is the smallest RNA (4s).



Function : At the time of protein synthesis, it acts as a carrier of amino-acids.

Structure : The structure of tRNA is most complicated.

Holley presented **Clover leaf model** of its structure. In two dimensional structure the tRNA appears clover leaf like but in three dimensional structure **(by Kim)** it appears inverted **L–shaped**.

* There are three nucleotides present in a particular sequence at 3' end of tRNA and that sequence is CCA. Whereas at 5′ end G(guanine) is present.
* 3' end is known as Acceptor end.
* tRNA accepts amino acids at acceptor end. Amino acid binds to 3' end by its – COOH group.
* The molecule of tRNA is folded and due to folding some complementary nitrogenous bases come across with each other and form hydrogen bonds.
* There are some places where hydrogen bonds are not formed, these places are known as loop.

Loops :–

There are some abnormal nitrogenous bases in the loops, that is why hydrogen bonds are not formed.

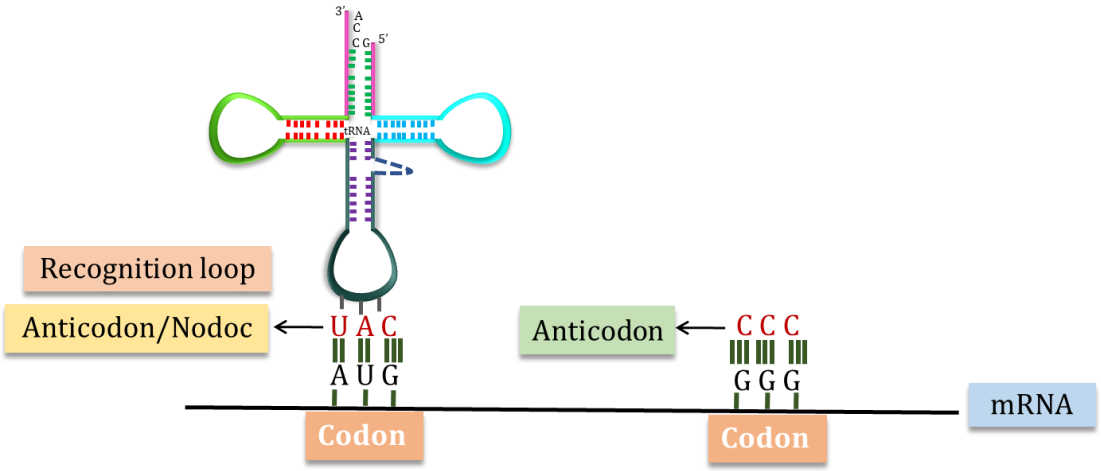
e.g. (i) Inosine (I) (ii) Pseudouracil (Ψ) (iii) Dihydrouridine (DHU)

(A) TΨC Loop or Attachment loop :–

This loop connects tRNA to the larger subunit of ribosome.

(B) Recognition Loop (Anticodon loop) :-

* This is the most specific loop of tRNA and different types of tRNA are different due to this loop. There is a specific sequence of three nucleotides called Anticodon, is present at the end of this loop.
* tRNA recognizes its place on mRNA with the help of Anticodon.
* The anticodon of tRNA recognises its complimentary sequence on mRNA. This complimentary sequence is known as codon.



(C) DHU Loop :–

* It is also known as Amino-acyl synthetase recognition loop. Amino-acyl synthetase is a specific type of enzyme, that activate a specific type of amino acid after activation this enzyme, amino acid attaches to the 3' end of tRNA.
* There are 20 types of enzymes for 20 types of amino acids.
* The function of DHU loop is to recognize this specific Aminoacyl synthetase enzyme.

(3) MESSENGER RNA (mRNA) :–

* The mRNA is 1 - 5% of the cell's total RNA.
  The name mRNA was given by **Jacob** and **Monod**.
* The mRNA is produced by genetic DNA in the nucleus.
* It is least stable RNA.

Question-1: Which one of the following loop is helped in attaching tRNA to larger subunit of ribosomes :-

(1) DHU - loop (2) TΨC loop (3) Anticodon loop (4) Minor loop

Answer: (2) TΨC loop

Question-2: Which form of RNA has a structure resembling clover leaf ?

(1) rRNA (2) hnRNA (3) mRNA (4) tRNA

Answer: (4) tRNA

Question-3: Which is soluble RNA :-

(1) hnRNA (2) rRNA (3) mRNA (4) tRNA

Answer: (4) tRNA

Question-4: A sequence of three consecutive bases in a tRNA molecule, which specifically binds to a complementary codon sequence in mRNA is known as -

(1) Triplet (2) Non - sense codon
(3) Anti codon (4) Termination codon

Answer: (3) Anti codon

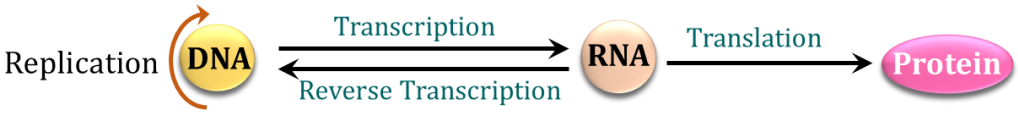
Question-5: Which may be attached with Adenine base in RNA -

(1) Guanine (2) Cytosine (3) Uracil (4) Thymine

Answer: (3) Uracil

CENTRAL DOGMA OF MOLECULAR BIOLOGY

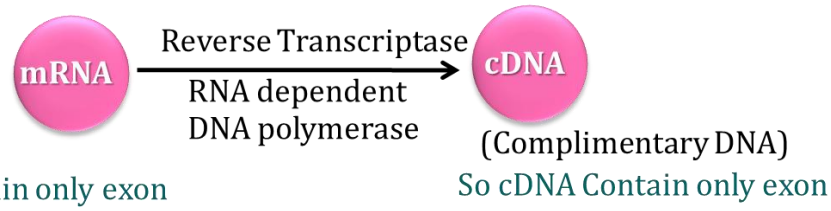
* Central dogma was given by Crick.
* The formation (production) of mRNA from DNA and then synthesis of protein from it, is known as Central Dogma.



* It means, it includes transcription and translation.

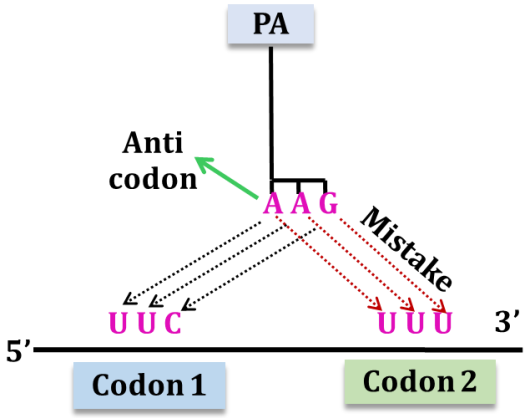
REVERSE TRANSCRIPTION :

* The formation of DNA from RNA is known as **Reverse - transcription**. It was discovered by **Temin** and **Baltimore** in Rous - sarcoma virus. So it is also called **Teminism**.
* ss–RNA of Rous–Sarcoma virus (Retro virus) produces dsDNA in host's cell with the help of enzyme reverse transcriptase (DNA–polymerase). This DNA is called cDNA (Complimentary DNA).



WOBBLE HYPOTHESIS :

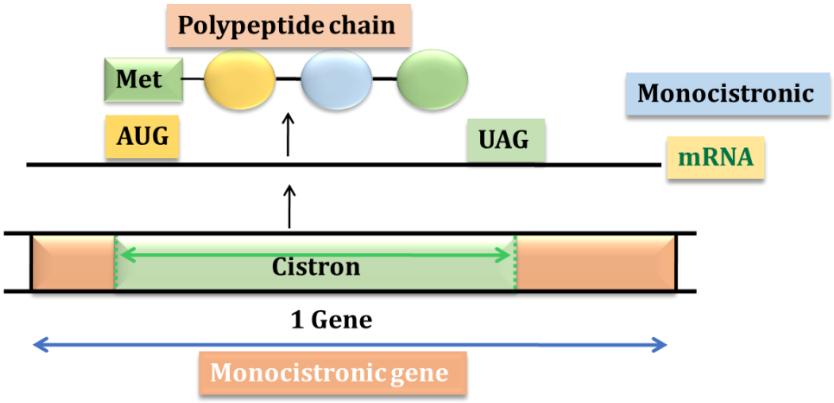
* It was propounded by CRICK.
* Normally an anticodon recognises only one codon, but sometimes an anticodon recognises more than one codon. This is known as Wobbling. Wobbling normally occurs for third nucleotide of codon.
* For e.g. anticodon AAG can recognise two codons i.e. UUU and UUC, both stands for phenyl alanine.



Types of mRNA – mRNA is of 2 types

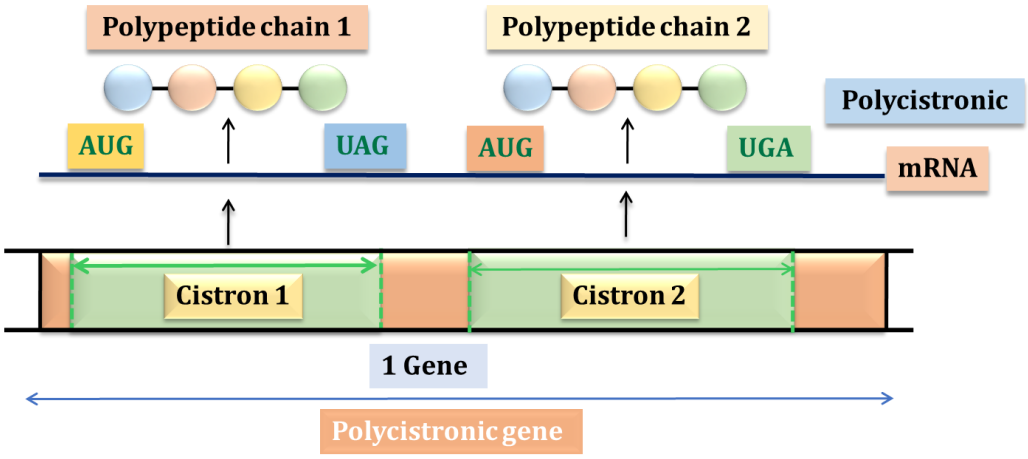
(1) MONOCISTRONIC :-

The mRNA in which genetic signal is present for the formation of only one polypeptide chain ***e.g.*** Eukaryotes.



(2) POLYCISTRONIC :-

The mRNA, in which genetic signal is present for the formation of more than one polypeptide chains ***e.g.*** Prokaryotes.



Question-1: The formation of mRNA from DNA

(1) Transcription (2) Central dogma (3) Translation (4) Replication

Answer: (1) Transcription

Question-2: Who among the following proposed central dogma :-

(1) Crick (2) Watson

(3) Her Govind Khorana (4) Nirenberg

Answer: (1) Crick

Question-3: Unidirectional flow of genetic information is known as :-

(1) Transcription (2) Central dogma (3) Translation (4) Replication

Answer: (2) Central dogma

Question-4: Central dogma includes :-

(1) Transcription (2) Translation (3) Both (1) and (2) (4) Reverse transcription

Answer: (3) Both (1) and (2)

SEARCH FOR GENETIC MATERIAL: TRANSFORMING PRINCIPLE AND ITS BIOCHEMICAL NATURE

The experiments given below prove that DNA is the genetic material.

(I) EVIDENCE FROM BACTERIAL TRANSFORMATION:

The transformation experiments conducted by Frederick Griffith in 1928, are of greater importance in establishing the nature of genetic material.

He used two strains of bacterium Diplococcus or Streptococcus pneumoniae or Pneumococcus i.e., S-III and R-II.

(i) Smooth (S) or capsulated type which have a mucous coat and produce shiny colonies. These bacteria are virulent and cause pneumonia.

(ii) Rough (R) or non-capsulated type in which mucous coat is absent and produce rough colonies. These bacteria are nonvirulent and do not cause pneumonia.

The experiment can be described in following four steps:

(a) Smooth type bacteria were injected into mice. The mice died as a result of pneumonia caused by bacteria.

Live S strain → injected into mice → Mice died

(b) Rough type bacteria were injected into mice. The mice lived and pneumonia was not produced

Live R strain → injected into mice → Mice survive

(c) Smooth type bacteria which normally cause disease were heat killed and then injected into the mice. The mice lived and pneumonia was not caused.

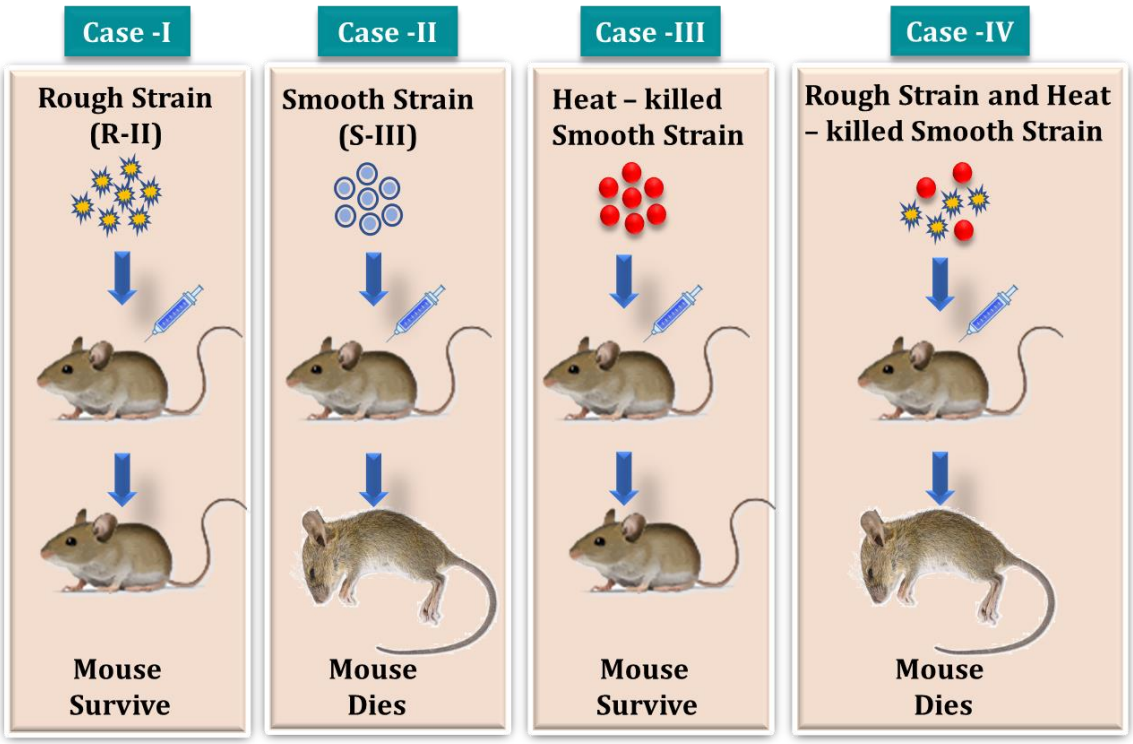
S strain (heat killed) → Injected into mice → Mice survive

(d) Rough type bacteria (living) and smooth type heat-killed bacteria (both known not to cause disease) were injected together into mice. The mice died due to pneumonia and virulent smooth type living bacteria could also be recovered from their bodies.

S strain (heat killed) + R strain (living) → injected into mice → Mice died

He concluded from fourth step of the experiment that some rough bacteria (nonvirulent) were transformed into smooth type of bacteria (virulent). This occurred perhaps due to absorption of some transforming substance by rough type bacteria from heat killed smooth type bacteria. This transforming substance from smooth type bacteria caused the synthesis of capsule which resulted in production of pneumonia and death of mice. Therefore, transforming principle appears to control genetic characters (for example, capsule as in this case).

However, the biochemical nature of genetic material was not defined from this experiment.

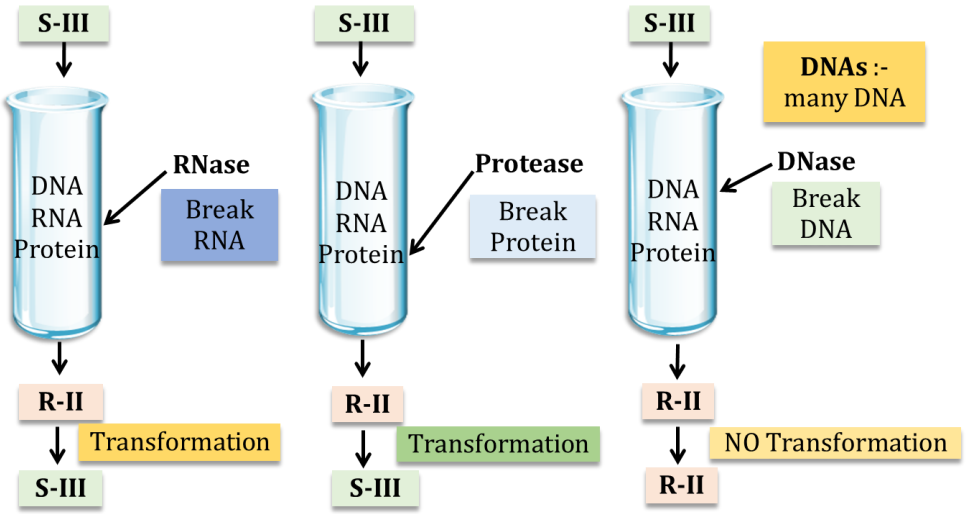


R-II strain transformed into S-III strain

(II) BIOCHEMICAL CHARACTERISATION OF TRANSFORMING PRINCIPLE :

Later, Avery, Macleod and McCarty (1944) repeated the experiment in vitro to identify the biochemical nature of transforming substance. They proved that this substance is DNA.

Pneumococcus bacteria cause disease when capsule is present. Capsule production is under genetic control.



In the experiments, rough type bacteria (non-capsulated and non-virulent) were grown in a culture medium to which DNA extract from smooth type bacteria (capsulated and virulent) was added. Later, the culture showed the presence of smooth type bacteria also in addition to rough type. This is possible only if DNA of smooth type was absorbed by rough type bacteria which developed capsule and became virulent. This process of transfer of characters of one bacterium to another by taking up DNA from solution is called **transformation**.

When DNA extract was treated with DNase (an enzyme which destroys DNA), transformation did not occur. The transformation occurs when proteases and RNases were used.
This clearly shows that DNA is the genetic material.

Question-1: Who among the following performed transforming experiment?

(1) Frederick Griffith (2) Watson (3) Crick (4) George Gamow

Answer: (1) Frederick Griffith

Question-2: What are the characteristics of rough pneumococci strain?

(1) Non-capsulated and pathogenic (2) Non-capsulated and non-pathogenic
(3) Capsulated and pathogenic (4) Capsulated and non-pathogenic

Answer: (2) Non-capsulated and non-pathogenic

Explanation:

Rough pneumococci strain can not cause pneumoniae as they do not have capsule.

Question-3: DNA transformation involves the transfer of naked DNA into a recipient cell.

(1) True (2) False

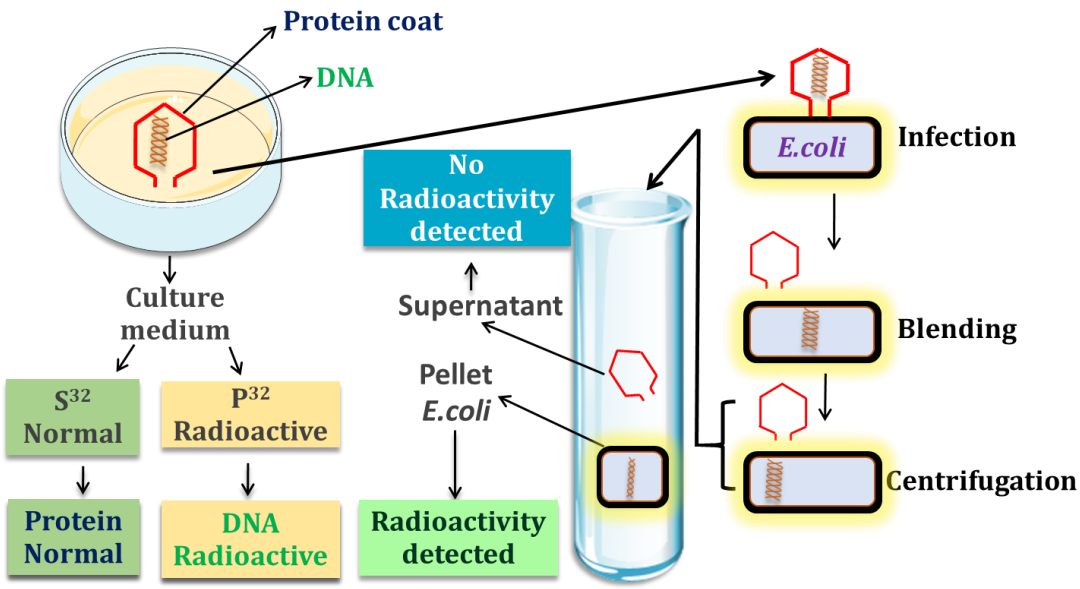
Answer: (1) True

(III) EVIDENCE FROM EXPERIMENTS WITH BACTERIOPHAGE :

T_2 bacteriophage is a virus that infects bacterium ***Escherichia coli*** and multiplies inside it. T_2 phage is made up of DNA and protein coat. Thus, it is the most suitable material to determine whether DNA or protein contains information for the production of new virus (phage) particles. Hershey and Chase (1952) demonstrated that only DNA of the phage enters the bacterial cell and, therefore, contains necessary genetic information for the assembly of new phage particle.

The functions of DNA and proteins could be found out by labelling them with radioactive tracers. DNA contains phosphorus but not sulphur. Therefore, phage DNA was labelled with P^{32} by growing bacteria infected with phage's in culture medium containing $^{32}PO_4$. Similarly, protein of phage contains sulphur but no phosphorus. Thus, the phage protein coat was labelled with S^{35} by growing bacteria infected with phage's in another culture medium containing $^{35}SO_4$. After the formation of labelled phage's. Three steps were followed, i.e., infection, blending, centrifugation.

1. **Infection :** Both type of labelled phage's were allowed to infect normally cultured bacteria in separate experiments.
2. **Blending :** These bacteria cells were agitated in a blender to break the contact between virus and bacteria.



3. **Centrifugation :** The virus particles were separated from the bacterium by spinning them in a centrifuge.

After the centrifugation the bacterial cells showed the presence of radioactive DNA labelled with P^{32} while radioactive protein labelled with S^{35} appeared on the outside of bacteria cells (i.e., in the medium).

Labelled DNA was also found in the next generation or phage. This clearly showed that only DNA enters the bacterial host and not the protein. DNA, therefore, is the infective part of virus and also carries all the genetic information. This provided the unequivocal proof that DNA is the genetic material.

Question-1: Hershey and chase experiment confirms that

(1) DNA is unequivocal Genetic material (2) RNA is unequivocal Genetic material

(3) Protein is unequivocal Genetic material (4) Carbohydrate is unequivocal Genetic material

Answer: (1) DNA is unequivocal Genetic material

Question-2: What is correct sequence process for Hershey and chase experiment?

(1) Infection → Blending → Centrifugation (2) Blending → Infection → Centrifugation

(3) Centrifugation → Infection → Blending (4) Blending → Centrifugation → Infection

Answer: (1) Infection → Blending → Centrifugation

Question-3: T_2 phage made up of

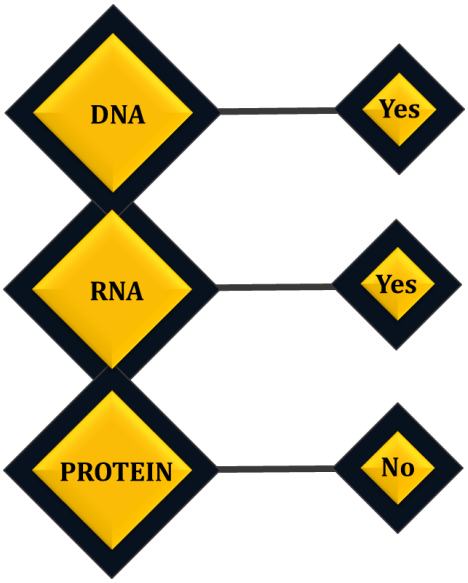
(1) DNA and protein coat (2) RNA and lipid coat

(3) RNA and protein coat (4) Only protein coat present

Answer: (1) DNA and protein coat

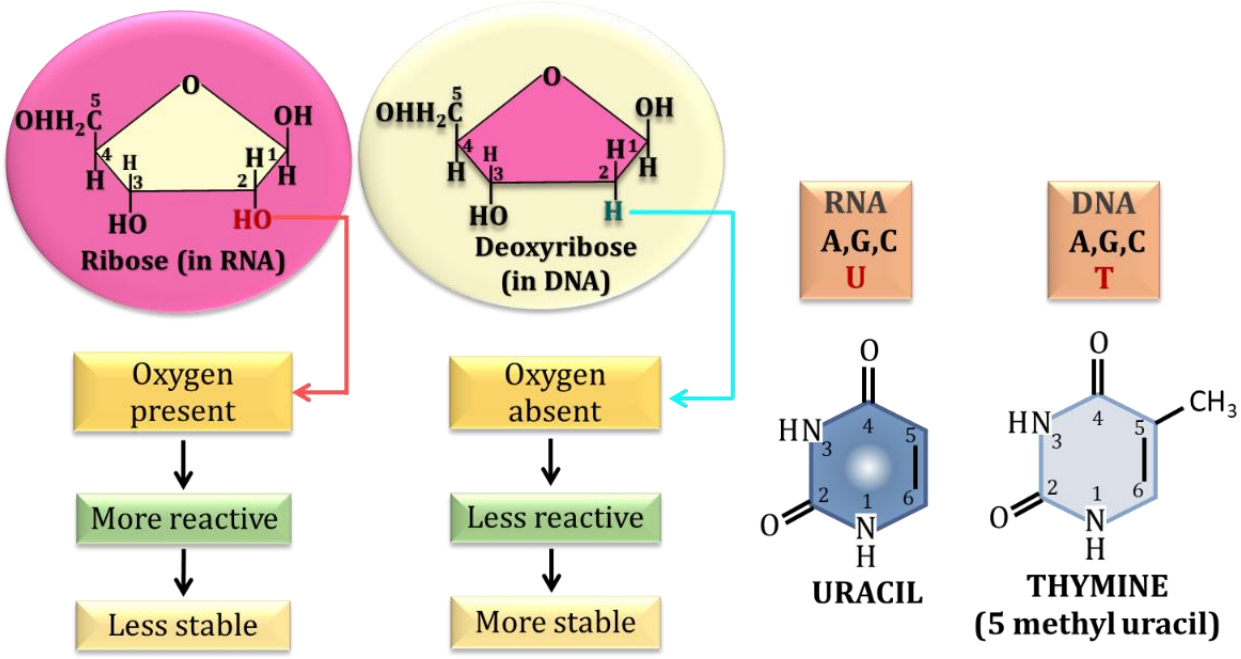
PROPERTIES OF GENETIC MATERIAL

Following are the properties and functions which should be fulfilled by a substance if it is to qualify as genetic material.

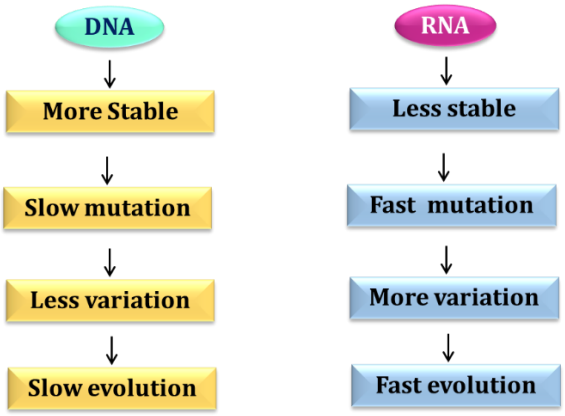


(1) The genetic material should be able to generate its own copy (replication). Both the nucleic acids (DNA and RNA) have the ability to direct their duplications. The other molecules in the living system, such as proteins fail to fulfil first criteria itself.

(2) It should chemically and structurally be stable. The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of the properties of genetic material was very evident in **Griffith's transforming principle** itself that heat, which killed the bacteria, at least did not destroy some of the properties of genetic material. This now can easily be explained in light of the DNA that the two strands being complementary if separated by heating come together, when appropriate conditions are provided. Further, 2'-OH group present at every nucleotide in RNA is a reactive group and makes RNA labile and easily degradable. RNA is also now known to be catalytic, hence reactive. Therefore, DNA chemically is less reactive and structurally more stable when compared to RNA. Therefore, among the two nucleic acids, the DNA is a better genetic material. In fact, the presence of thymine at the place of uracil also confers additional stability to DNA.

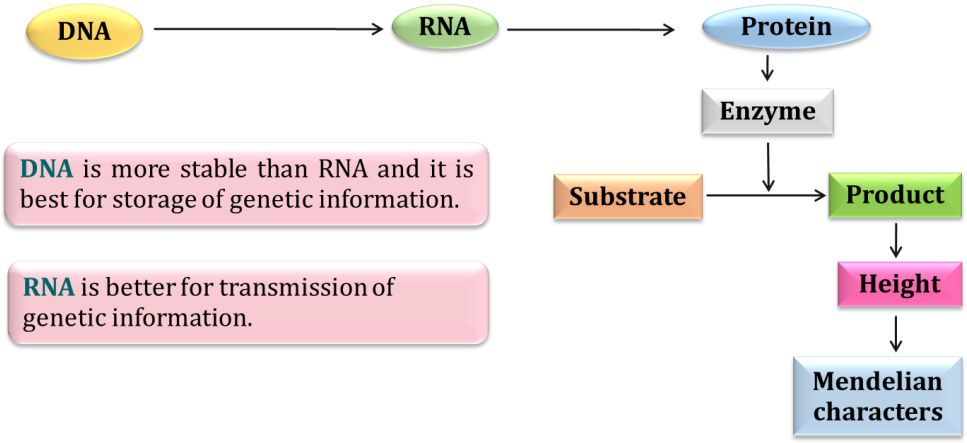


(3) The genetic material should also be capable of undergoing mutations. Both DNA and RNA are able to mutate. In fact, RNA being unstable, mutate at a faster rate. Consequently, viruses having RNA genome and having shorter life span mutate and evolve faster.



(4) The genetic material should be able to transmit faithfully to the next generation, as Mendelian characters. RNA can directly code for the synthesis of proteins, hence can easily express the characters. DNA, however, is dependent on RNA for synthesis of proteins. The protein synthesising machinery has evolved around RNA.

The above discussion indicate that both RNA and DNA can function as genetic material, but DNA being more stable is preferred for storage of genetic information. For the transmission of genetic information, RNA is better.



Question-1: Which of the following property fulfilled by the genetic material?

(1) Occurrence of replication (2) should be able to express itself
(3) Chemically and structurally stable (4) All above

Answer: (4) All above

Question-2: Stability is not property of genetic material?

(1) True (2) False

Answer: (2) False

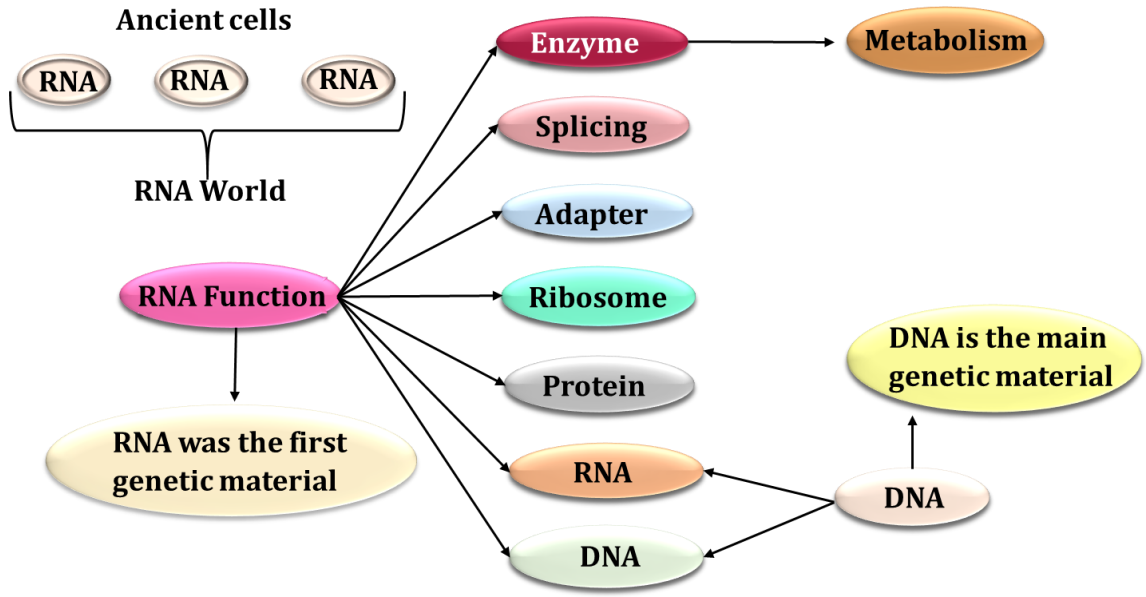
Question-3: Which one of the following is better for transmission of genetic information?

(1) DNA (2) RNA (3) Nucleotide (4) Carbohydrate

Answer: (2) RNA

RNA WORLD

RNA was the first genetic material. There are evidences to suggest that essential life processes, such as metabolism, translation, splicing etc. evolved around RNA. RNA used to act as a genetic material as well as a catalyst, there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes (e.g., splicing) RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable. DNA being double stranded and having complementary strand further resists changes by evolving a process of repair. RNA is adapter, structural molecule and in some cases catalytic. Thus RNA is better material for transmission of information.



Question-1: Which of the following is first genetic material-

(1) RNA (2) DNA (3) Protein (4) Carbohydrate

Answer: (1) RNA

Question-2: DNA has evolved from

(1) RNA (2) Protein (3) Carbohydrate (4) Lipids

Answer: (1) RNA

Question-3: According to RNA world hypothesis primary living substance is

(1) DNA (2) Enzymes (3) Lipids (4) RNA

Answer: (4) RNA

Question-4: Which of the following property is not belong to RNA

(1) Less stable than DNA (2) More stable than DNA

(3) Catalytic property (4) Biochemically active

Answer: (2) More stable than DNA

REPLICATION OF DNA (INTRODUCTION AND EXPERIMENTS)

* While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows:
* It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material. (Watson and Crick, 1953).
* The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This scheme was termed as semiconservative DNA replication
* DNA capable of self-duplication.
* All living beings have the capacity to reproduce because of this characteristic of DNA
* DNA replication takes place in **"S - Phase"** of the cell cycle. At the time of cell division, it divides in equal parts in the daughter cells.

SEMI CONSERVATIVE MODE OF DNA REPLICATION

Semi conservative mode of DNA replication was first proposed by Watson & Crick. Later on it was experimentally proved by ***Meselson & Stahl (1958)*** *in* ***E. Coli*** and ***Taylor*** in ***Vicia faba*** **(1958)**. To prove this method, Taylor used Radiotracer Technique in which Radioisotopes (tritiated thymidine = ${}_1H^3$) were used. Meselson and Stahl used heavy isotope of nitrogen (N^{15}).

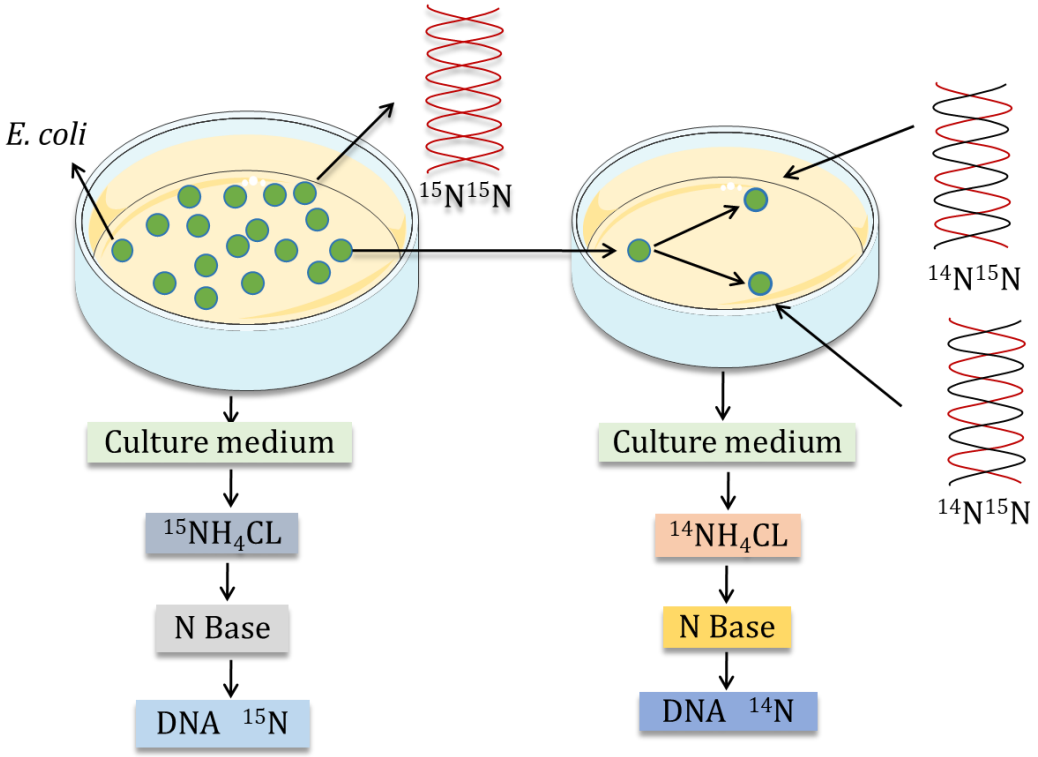
MATTHEW MESELSON AND FRANKLIN STAHL PERFORMED THE FOLLOWING EXPERIMENT IN 1958 :

(i) They grew ***E.coli*** in a medium containing $^{15}NH_4Cl$ (^{15}N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that ^{15}N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds). This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a Cesium Chloride (CsCl) density gradient (Please note that ^{15}N is not a radioactive isotope, and it can be separated from ^{14}N only based on densities).

(ii) Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices.

The various samples were separated independently on CsCl gradients to measure the densities of DNA.

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from ^{15}N to ^{14}N medium [that is after 20 minutes; E. coli divides in 20 minutes] had a hybrid or intermediate density. DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of light DNA.



Question-1: In Meselson and Stahl's experiment, heavy isotope ^{15}N was used in the form of :-

(1) $NaNO_3$ (2) NH_4Cl

(3) KNO_3 (4) NH_4NO_3

Answer: (2) NH_4Cl

Question-2: DNA replication is :-

(1) Conservative (2) Semi-conservative

(3) Dispersive (4) Continuous

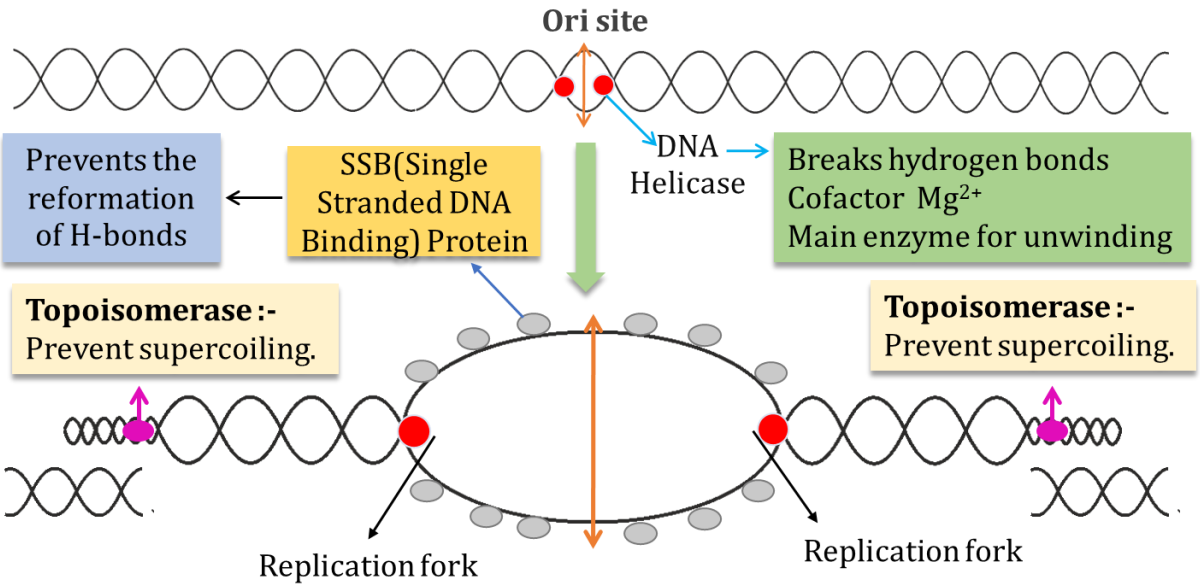
Answer: (2) Semi-conservative

MECHANISM OF DNA REPLICATION

The following steps are included in DNA replication :-

(1) UNZIPPING (UNWINDING) :–

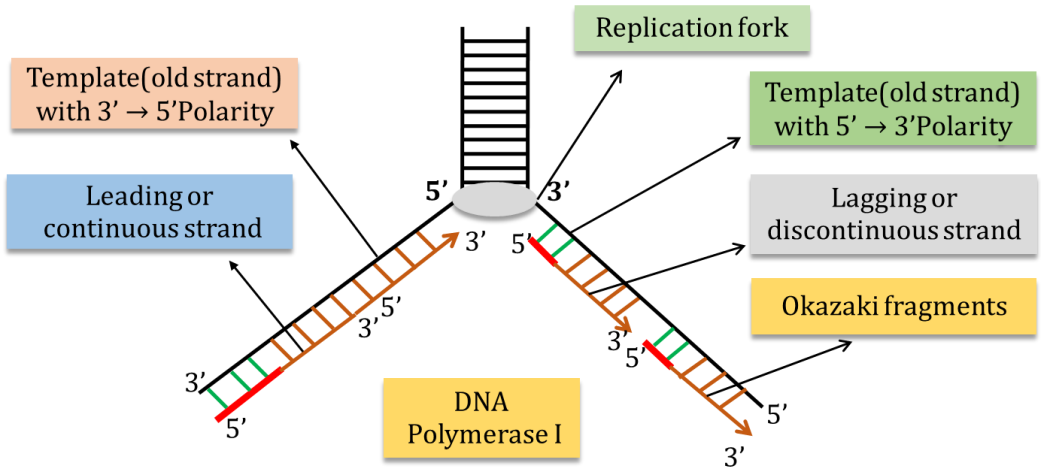
* The separation of 2 chains of DNA is termed as unzipping. And it takes place due to the breaking of Hydrogen bonds. The process of unzipping starts at a certain specific point which is termed as initiation point or origin of replication. In prokaryotes there occurs only one origin of replication but in eukaryotes there occur many origin of replication i.e. unzipping starts at many points simultaneously. At the place of origin the enzyme responsible for unzipping (breaking the hydrogen bonds) is Helicase (= Swivels). In the process of unzipping Mg^{+2} acts as cofactor.
* SSB (single stranded DNA binding protein) prevents the reformation of H-bonds.
* Topoisomerase (in prokaryotes also called as DNA gyrase) releases the tension arises due to supercoiling.



Note **:** The process of DNA replication takes a few minutes in prokaryotes and a few hours in Eukaryotes.

(2) FORMATION OF NEW CHAIN :-

* To start the synthesis of new chain, special type of RNA is required which is termed as RNA Primer. The formation of RNA primer is catalysed by an enzyme - RNA Polymerase (primase). Synthesis of RNA Primer takes place in 5' $\rightarrow$ 3' direction.
  After the formation of new chain, this RNA is removed. For the formation of new chain Nucleotides are obtained from Nucleoplasm. In the nucleoplasm, Nucleotides are present in the form of triphosphates like dATP, dGTP, dCTP, dTTP etc.



* During replication, the 2 phosphate groups of all nucleotides are separated. In this process energy is yielded which is consumed in DNA replication.
* Energetically replication is a very expensive process. Deoxyribonucleoside triphosphates serve dual purposes in addition to acting as substrates they provide energy for polymerisation.
* The formation of new chain always takes place in 5' - 3' direction. As a result of this, one chain of DNA is continuously formed and it is termed as Leading strand. The formation of second chain begins from the centre and not from the terminal points, so this chain is discontinuous and is made up of small segments called Okazaki Fragments. This discontinuous chain is termed as Lagging strand. Ultimately all these segments joined together and a complete new chain is formed.
* The Okazaki fragments are joined together by an enzyme DNA Ligase.
* The formation of new chains is catalysed by an enzyme DNA Polymerase. In prokaryotes it is of 3 types:

(1) DNA - POLYMERASE I :-

This was discovered by KORNBERG (1957). So it is also called as 'Kornberg's enzyme'. Kornberg also synthesized DNA first of all, in the laboratory. This enzyme functions as exonuclease. It separates RNA - primer from DNA and also fills the gap. It is also known as DNA-repair enzyme.

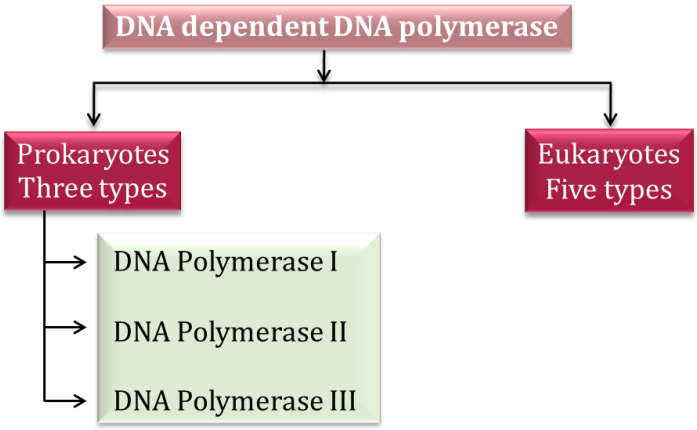
(2) DNA - POLYMERASE II :-

It is least reactive in replication process. It is also helpful in DNA-repairing in absence of DNA-polymerase-I and DNA polymerase-III

(3) DNA - POLYMERASE III :-

This is the main enzyme in DNA - Replication. It is most important. The larger chains are formed by this enzyme. This is also known as Replicase.

* In the semi conservative mode of replication each daughter DNA molecule receives one chain of polynucleotides from the mother DNA - molecule and the second chain is synthesized.



	DNA Polymerase I	DNA Polymerase II	DNA Polymerase III
	Discovered by Kornberg	Least reactive in replication	Main enzyme for replication
5' → 3' Polymerisation (Chain formation)	Form short chain (gap filling)	Form short chain	Form long chain
Remove RNA primer	Yes	No	No
DNA repair	Yes	Yes	No
5' → 3' exonuclease	Yes	No	No
3' → 5' exonuclease	Yes	Yes	Yes

Special Point :

* All DNA polymerase I, II and III enzymes have 5'-3' polymerisation activity and 3'-5' exonuclease activity.
* DNA polymerase I also has 5'-3' exonuclease activity.
* Any failure in cell division after DNA replication result into polyploidy.
* Difference between DNAs and DNase is that DNAs means many DNA and DNase means DNA digestive enzymes.

NCERT Insider :

In living cells, such as *E. coli*, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-dependent DNA polymerase, since it uses a DNA template to catalyse the polymerisation of deoxynucleotides. These enzymes are highly efficient enzymes as they have to catalyse polymerisation of a large number of nucleotides in a very short time. *E. coli* that has only 4.6×10^6 bp (compare it with human whose diploid content is 6.6×10^9 bp), completes the process of replication within 18 minutes; that means the average rate of polymerisation has to be approximately 2000 bp per second. Not only do these polymerases have to be fast, but they also have to catalyse the reaction with high degree of accuracy. Any mistake during replication would result into mutations.

Question-1: The enzyme which shows polymerising activity in 5' → 3' direction is -

(1) DNA polymerase III (2) DNA polymerase II

(3) DNA polymerase I (4) All of these

Answer: (4) All of these

Question-2: DNA polymerase I is involved in -

(1) Removal of RNA primer (2) Filling of gap

(3) Joining of Okazaki fragments (4) Both (1) and (2)

Answer: (4) Both (1) and (2)

Question-3: Which of the following enzyme is used to join DNA fragments :-

(1) Exonuclease (2) Endonuclease (3) Ligase (4) DNA polymerase

Answer: (3) Ligase

Question-4: Okazaki fragments are synthesised on :-

(1) Leading strands of DNA only (2) Lagging strands of DNA only

(3) Both leading and lagging strands of DNA (4) Complementary DNA

Answer: (2) Lagging strands of DNA only

Question-5: DNA replication includes -

(1) DNA ligase (2) DNA polymerase and ligase

(3) RNA polymerase and ligase (4) All of these

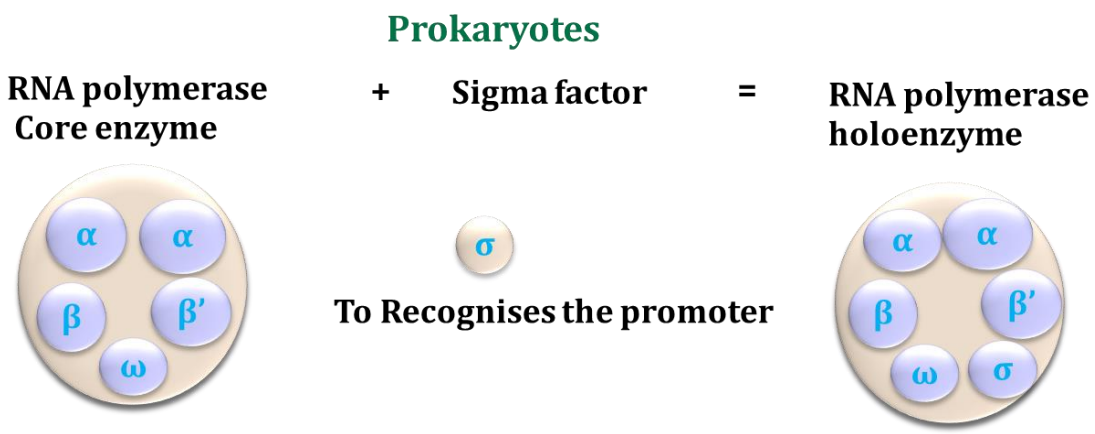
Answer : (4) All of these

TRANSCRIPTION

* The process of copying genetic information from one strand of the DNA into RNA is termed as transcription. Out of two strand of DNA only one strand participates in transcription and called ''Antisense strand'' or "Template strand".
* If both strands act as a template during transcription they would code for RNA molecule with different sequence and If they code for proteins the sequence of amino acid in these proteins would be different and another reason that if the two RNA molecule produced they would be complementary to each other and form a dsRNA which prevents translation of RNA.
* A gene is defined as the functional unit of inheritance. It is difficult to literally define a gene in terms of DNA sequence, because the DNA sequence coding for tRNA or rRNA molecule is also define a gene (But information of protein is present on the DNA segment which code mRNA).
* The segment of DNA which contains signal for the synthesis of one polypeptide is known as ''Cistron''.
* RNA polymerase enzyme is involved in transcription. In eukaryotes there are three types of RNA polymerases.

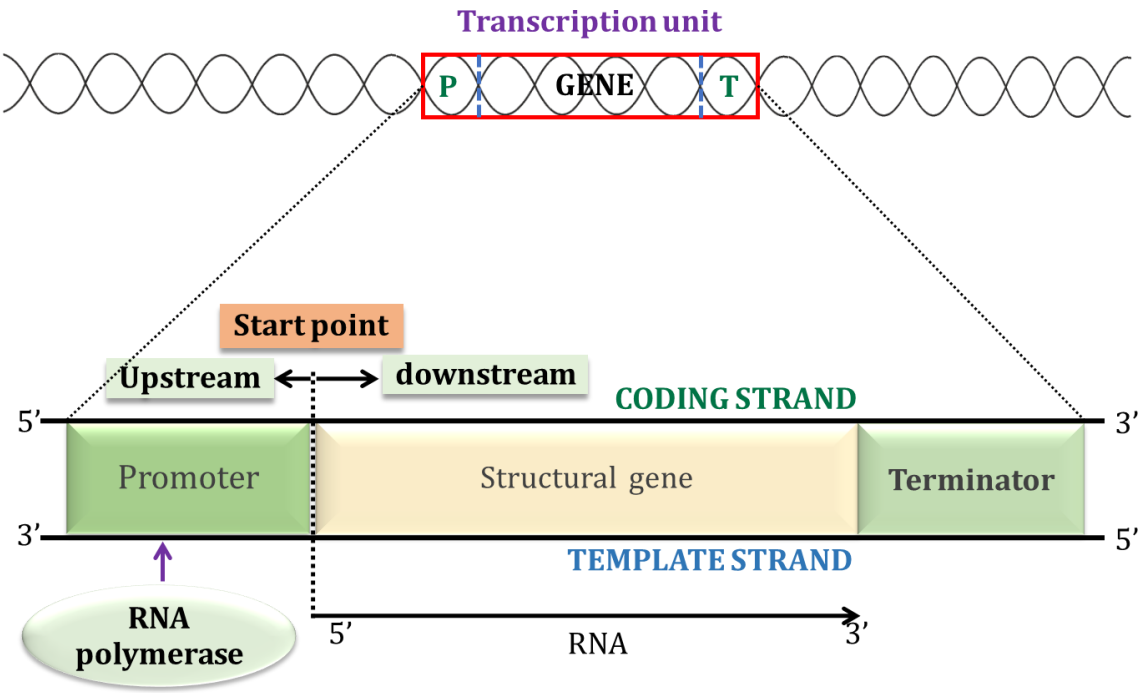
RNA polymerase–I for 28s rRNA, 18s rRNA, 5.8s rRNA synthesis.
RNA polymerase–II for hnRNA synthesis (Precursor of m-RNA).
RNA polymerase–III for tRNA, 5s rRNA, SnRNA synthesis.

* Prokaryotes have only one type of RNA polymerase which synthesizes all types of RNAs.
* RNA polymerase (Core enzyme) of ***E. coli*** has five polypeptide chains β, β′, α, α and ω.
* σ polypeptide chain is also known as σ factor (sigma factor).
* Core enzyme + Sigma factor ⇒ RNA Polymerase holoenzyme



A transcription unit in DNA is defined primarily by three regions in the DNA :-

(i) A promoter
(ii) The structural gene
(iii) A terminator



NCERT INSIDER :

The promoter and terminator flank the structural gene in a transcription unit. The promoter is said to be located towards 5'-end (upstream) of the structural gene (the reference is made with respect to the polarity of coding strand). It is a DNA sequence that provides binding site for RNA polymerase, and it is the presence of a promoter in a transcription unit that also defines the template and coding strands. By switching its position with terminator, the definition of coding and template strands could be reversed. The terminator is located towards 3'-end (downstream) of the coding strand and it usually defines the end of the process of transcription. There are additional regulatory sequences that may be present further upstream or downstream to the promoter. Some of the properties of these sequences shall be discussed while dealing with regulation of gene expression.

Question-1: Out of two strands of DNA only one strand participates in transcription and called

(1) Antisense strand
(2) Template strand
(3) Both (1) and (2)
(4) Coding strand

Answer: (3) Both (1) and (2)

Question-2: The segment of DNA, which contains signal for the synthesis of one polypeptide is known as

(1) Cistron
(2) Muton
(3) Recon
(4) All of these

Answer: (1) Cistron

Question-3: RNA polymerase (Core enzyme) of E. Coli has

(1) Five polypeptide chains
(2) Six polypeptide chains
(3) Three polypeptide chains
(4) Seven polypeptide chains

Answer: (1) Five polypeptide chains

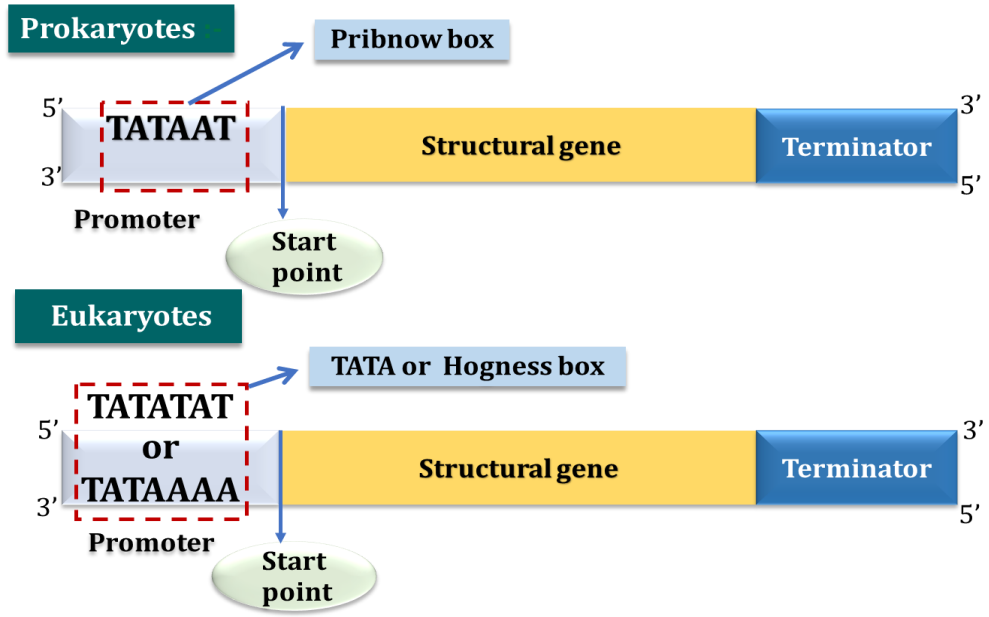
TRANSCRIPTION

Following steps are present in transcription –

(1) INITIATION :–

* DNA has a ''Promoter site'' where RNA polymerase binds and a ''Terminator site'' where transcription stops.
* Sigma factor (σ) recognises the promoter site of DNA.
* With the help of sigma factor RNA polymerase enzyme attached to a specific site of DNA called **''Promoter site''**.
* In prokaryotes before the 10 N_2 base from **''Starting point''** a sequence of 6 base pairs (TATAAT) is present on DNA, which is called **''Pribnow box''**.

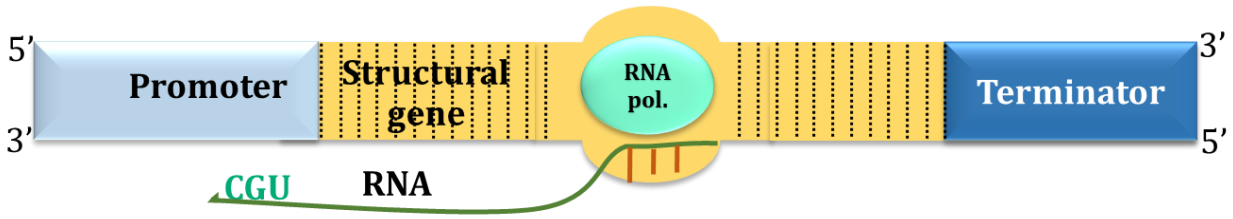
* In eukaryotes before the 20 N_2 base from **''Starting point''** a sequence of 7 base pairs (TATAAAA) or (TATATAT) is present on DNA which is called **"TATA box** or **Hogness box"**



* At start point RNA polymerase enzyme breaks H–bonds between two DNA strands and separates them.
* One of them strand takes part in transcription. Transcription proceeds in 5' → 3' direction.
* Ribonucleoside triphosphates come to lie opposite complementary nitrogen bases of anti sense strand.
* These Ribonucleotides present in the form of triphosphate ATP, GTP, UTP and CTP. When they are used in transcription, pyrophosphatase hydrolyse two phosphates from each activated nucleotide. This releases energy. This energy is used in the process of transcription.
*

(2) ELONGATION :–

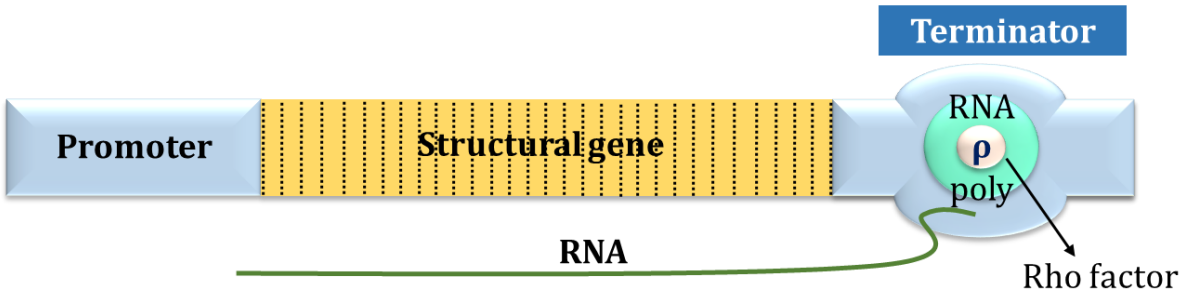
* RNA polymerase enzyme establishes phosphodiester bond between adjacent ribonucleotides.
* Sigma factor separates and RNA polymerase moves along the anti sense strand till it reaches terminator site.

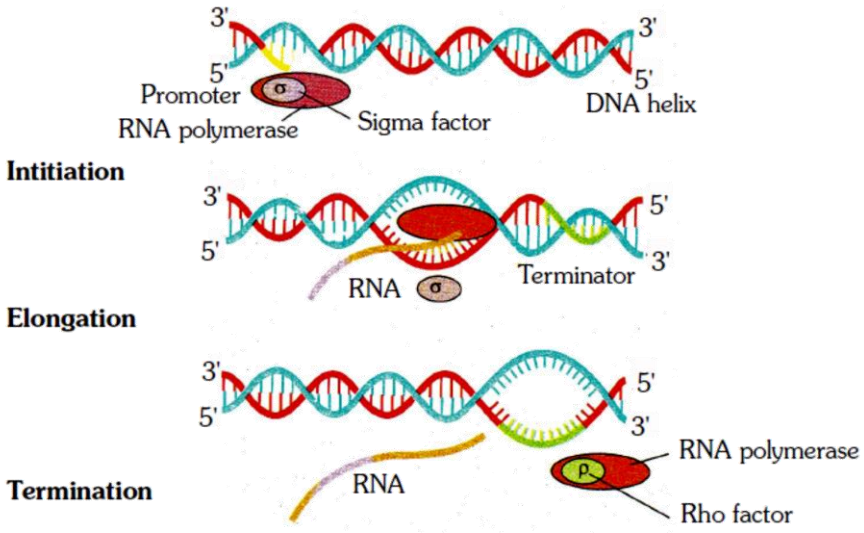


(3) TERMINATION :–

* When RNA polymerase enzyme reaches at terminator site, it separates from DNA template.
* In most cases RNA polymerase enzyme can recognise the 'Terminator site' and stop the synthesis of RNA chain, but in prokaryotes, it recognises the terminator site with the help of **Rho factor (ρ factor)**.

* Rho (ρ) factor is a specific protein which helps RNA polymerase enzyme to recognise the terminator site.





Process of Transcription in Bacteria

NCERT Insider :

Defining a cistron as a segment of DNA coding for a polypeptide, the structural gene in a transcription unit could be said as monocistronic (mostly in eukaryotes) or polycistronic (mostly in bacteria or prokaryotes). In eukaryotes, the monocistronic structural genes have interrupted coding sequences – the genes in eukaryotes are split. The coding sequences or expressed sequences are defined as exons. Exons are said to be those sequence that appear in mature or processed RNA. The exons are interrupted by introns. Introns or intervening sequences do not appear in mature or processed RNA. The split-gene arrangement further complicates the definition of a gene in terms of a DNA segment.

Inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as regulatory genes, even though these sequences do not code for any RNA or protein.

Question-1: Where transcription stops.

(1) Promoter site (2) Terminator site (3) Structural gene (4) Initiation site

Answer: (2) Terminator site

Question-2: The promoter site of DNA is recognised by

(1) Sigma factor (2) β factor (3) ψ factor (4) Rho factor

Answer: (1) Sigma factor

Explanation:

Sigma factor (σ) recognises the promoter site of DNA. With the help of sigma factor RNA polymerase enzyme attached to a specific site of DNA called "***Promoter site***".

Question-3: A box present in prokaryotes before the 10 N-base from "*Starting point*" a sequence of 6 base DNA, which is called

(1) Pribnow box (2) TATA BOX (3) Hogness box (4) All of these

Answer: (1) Pribnow box

Explanation:

In prokaryotes before the 10 N_2 base from "*Starting point*" a sequence of 6 base pairs (TATAAT) is present on DNA, which is called "***Pribnow box***".

Question-4: Which of the following recognises, terminator site in prokaryotes :-

(1) Rho factor (2) Beta factor (3) Sigma factor (4) ψ factor

Answer: (1) Rho factor

Explanation:

In prokaryotes, it recognises the terminator site with the help of Rho factor (ρ factor).

Rho (ρ) factor is a specific protein which helps RNA polymerase enzyme to recognise the terminator site.

POST TRANSCRIPTIONAL MODIFICATION

SPLIT GENE :

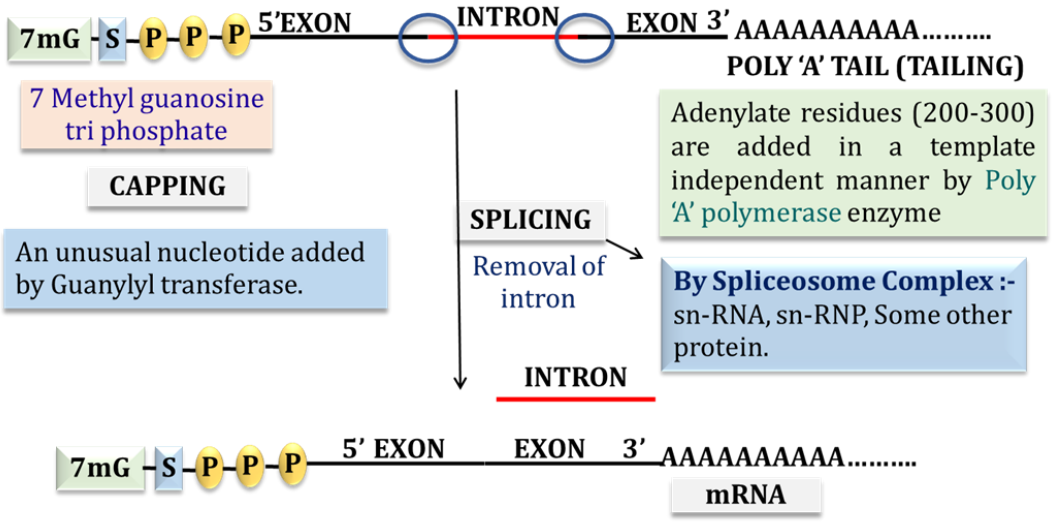
Discovered by **Sharp** and **Roberts**. They were awarded Nobel Prize in 1993. Gene which contains non-functional part along with functional part is known as split gene. Non-functional part is called **intron** and functional part is called **exon**. By transcription split gene produces a RNA which contains coding and non-coding sequence and called hn RNA (Hetero genous nuclear RNA). This hn RNA is unstable. Now 7 methyl guanosines is added to its 5' end, and a cap like structure is formed. It is called **capping** and 200-300 nucleotides of adenylic acid are added in template independent manner to its 3' end, which is called **poly 'A' tail**, Now it becomes stable. By the process of **RNA splicing** hn–RNA produces functional m-RNA that is exonic RNA. In RNA splicing non-coding parts is removed with the help of spliceosome complex and coding parts join together with the help of RNA ligase. Some specific proteins are also helpful in RNA - splicing called **'Small nuclear ribonucleoprotein' or 'SnRNP' or 'Snurps'**. These SnRNP proteins combine with some other proteins and SnRNA to form **spliceosome complex**. This spliceosome complex uses energy of ATP

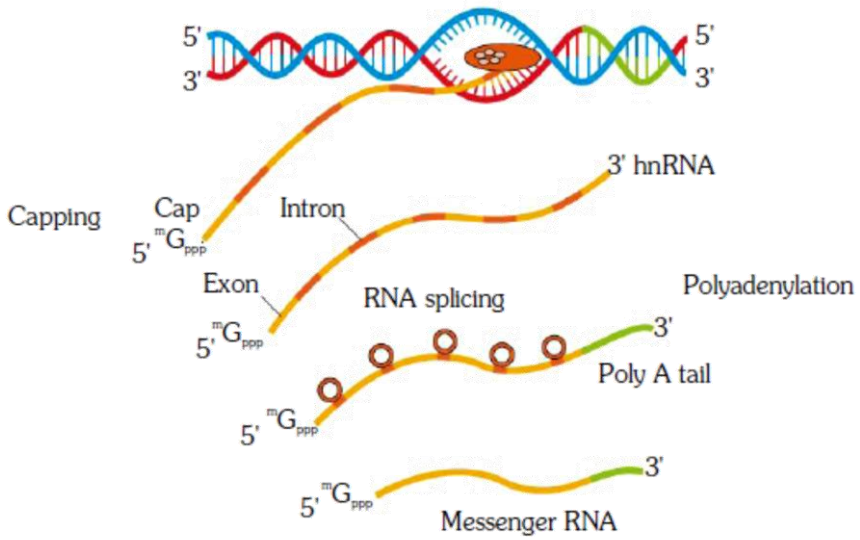
to cut the RNA, releases the non-coding part and joins the coding-part to produce functional RNA. Non-coding part of hnRNA remained inside the nucleus and not translated in to protein. Only coding part moves from nucleus to cytoplasm and gets translated into protein.

Mostly Eukaryotic genes are example of split gene, **but gene which forms histone and interferon protein are non-split gene**.

Mostly prokaryotic genes are example of non-split gene.

- In eukaryotes after transcription splicing process also occurred.
- The split gene represent an ancient (primitive) feature of gene.
- Presence of intron is a primitive character.
- The splicing process represent the dominance of RNA world.





Process of Transcription in Eukaryotes

Question-1: Gene which contains non-functional part along with functional part is known as

(1) Non-split gene (2) Split gene (3) Regulator gene (4) Operator gene

Answer: (2) Split gene

Question-2: RNA which contains coding and non-coding sequence are called :-

(1) RNA (2) m RNA (3) hnRNA (4) zDNA

Answer: (3) hnRNA

Explanation:

Transcription split gene produces a RNA which contains coding and non-coding sequence and called hnRNA (Hetero genous nuclear RNA). This hnRNA is unstable

Question-3: Which one of the following enzymes add cap at 5' end: -

(1) Guanylyl transferase (2) Oxidoreductase

(3) Kinase (4) Phosphatase

Answer: (1) Guanylyl transferase

GENETIC CODE

* Term Given by ***George Gamow***.
* Discovered by Nirenberg, Matthaei and Khorana.
* The relationship between the sequence of amino acids in a polypeptide chain and nucleotide sequence of DNA or mRNA is called ***genetic code***.
* There occur 20 types of amino acids which participate in protein synthesis. DNA contains information for the synthesis of any types of polypeptide chain. In the process of transcription, information is transfered from DNA to mRNA in the form of complementary N_2–base sequences.
* mRNA contains code for each amino acid and it is called codon. A codon is the nucleotide sequence on mRNA which codes for particular amino acid; whereas the genetic code is the sequence of nucleotides on mRNA molecule, which contains information for the synthesis of polypeptide chain.

TRIPLET CODE: –

* The main problem of genetic code was to determine the exact number of nucleotides in a codon which codes for one amino acid.
* There are four types of N_2–bases in mRNA (A, U, G, C) for 20 types of amino acids.
* If genetic code is ***singlet*** i.e. codon is the combination of only one nitrogen base, then only four codons are possible A, C, G and U. These are insufficient to code for 20 types of amino acids.

N base = 4 Amino acids = 20	Total codon	Coded amino acids	Remaining amino acids
Singlet (one N-base code one amino acid)	$4^1 = 4$	4	16
Doublet (two N-bases code one amino acid)	$4^2 = 16$	16	4
Triplet (three N-bases code one amino acid)	$4^3 = 64$	20	0
Tetraplate (four N-bases code one amino acid)	$4^4 = 256$	20	0

- Singlet code = $4^1 = 4 \times 1 = 4$ codons
- If genetic code is ***doublet*** (i.e. codon is the combination of two nitrogen bases) then 16 codons are formed.
- Doublet code = $4^2 = 4 \times 4 = 16$ codons.
- 16 codons are insufficient for 20 amino acid

AA	AC	AG	AU
CC	CA	CG	CU
GG	GA	GC	GU
UU	UA	UG	UC

Doublet Code: $4 \times 4 = 16$ codons

* Gamow (1954) pointed out the possibility of three letters code (Triplet code).
* Genetic code is triplet i.e. one codon consists of three nitrogen bases

  Triplet code = $4^3 = 4 \times 4 \times 4 = 64$ codons
* In this case there occurs 64 codons in dictionary of genetic code.
* 64 codons are sufficient to code 20 types of amino acids.
* The chemical method developed by Har Gobind Khorana was instrumental in synthesising RNA molecules with defined combinations of bases (homopolymers and copolymers). Marshall Nirenberg's cell-free system for protein synthesis finally helped the code to be deciphered. Severo Ochoa enzyme (polynucleotide phosphorylase) was also helpful in polymerising RNA with defined sequences in a template independent manner (enzymatic synthesis of RNA).

First (5') letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third (3') letter
U	UUU, UUC: Phe (F)	UCU, UCC, UCA, UCG: Ser (S)	UAU, UAC: Tyr (Y)	UGU, UGC: Cys (C)	U
	UUA, UUG: Leu (L)		UAA: Stop (terminator); UAG: Stop (terminator)	UGA: Stop (terminator); UGG: Trp (W)	C
					A
					G
C	CUU, CUC, CUA, CUG: Leu (L)	CCU, CCC, CCA, CCG: Pro (P)	CAU, CAC: His (H)	CGU, CGC, CGA, CGG: Arg (R)	U
			CAA, CAG: Gln (Q)		C
					A
					G
A	AUU, AUC, AUA: Ile (I)	ACU, ACC, ACA, ACG: Thr (T)	AAU, AAC: Asn (N)	AGU, AGC: Ser (S)	U
	AUG: Met (M) (initiator)		AAA, AAG: Lys (K)	AGA, AGG: Arg (R)	C
					A
					G
G	GUU, GUC, GUA, GUG: Val (V)	GCU, GCC, GCA, GCG: Ala (A)	GAU, GAC: Asp (D)	GGU, GGC, GGA, GGG: Gly (G)	U
			GAA, GAG: Glu (E)		C
					A
					G

CHARACTERISTICS OF GENETIC CODE :–

(i) TRIPLET IN NATURE: –

A codon is composed of three adjacent nitrogen bases which specifies one amino acid in polypeptide chain.

For Ex. :

- In mRNA if there are total 90 N_2 – bases.
- Then this mRNA determines 30 amino acids in polypeptide chain.
- In above example, number of nitrogen bases are 90, so codons $\Rightarrow$ 30. And 30 codons decide 30 amino acids in polypeptide chain.

(II) UNIVERSALITY: –

The genetic code is applicable universally. The same genetic code is present in all kinds of living organism including viruses, bacteria, unicellular and multicellular organisms.

(iii) UNAMBIGUOUS AND SPECIFIC: –

* Genetic code is unambiguous i.e. one codon specifies only one amino acid and not any other.

* In this case one codon never, code for two different amino acids. Exception GUG codon which codes both valine and methionine amino acids.

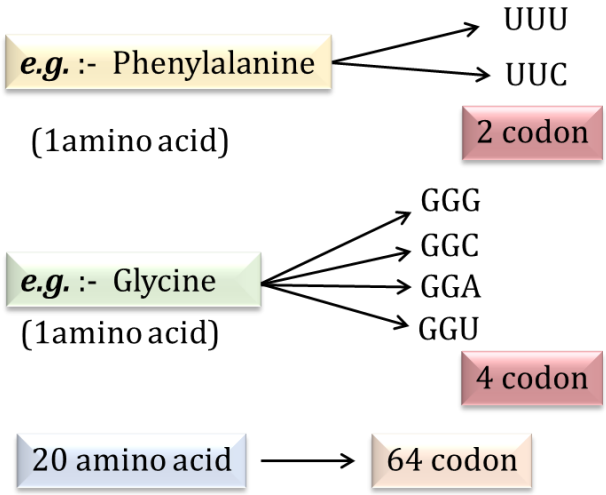
(iv) NON–OVERLAPPING: – A nitrogen base is a constituent of only one codon.

(v) COMMA LESS: –

* There is no punctuation (comma) between the adjacent codon i.e. each codon is immediately followed by the next codon.
* If a nucleotide is deleted or added, the whole genetic code read differently.
* A polypeptide chain having 50 amino acids shall be specialized by a linear sequence of 150 nucleotides. If a nucleotide is added in the middle of this sequence, the first 25 amino acids of polypeptide will be same but next 25 amino acids will be different.

(vi) DEGENERACY OF GENETIC CODE :–

* There are 64 codons for 20 types of amino acids, so most of the amino acids (except two) can be coded by more than one codon. Single amino acid coded by more than one codon is called ''Degeneracy of genetic code''. This incident was discovered by Baurnfield and Nirenberg.
* Only two amino acids *Tryptophan* and *Methionine* are specified by single codon.



All the other amino acids are specified or coded by 2 to 6 codons.

* Leucine, serine and arginine are coded or specified by 6–codons.

Leucine = CUU, CUC, CUA, CUG, UUA & UUG
Serine = UCU, UCC, UCA, UCG, AGU, AGC
Arginine = CGU, CGC, CGA, CGG, AGA, AGG

CHAIN INITIATION AND CHAIN TERMINATION CODON: –

* Polypeptide chain synthesis is signalled by two initiation codons AUG or GUG.
* AUG codes methionine amino acid in eukaryotes and in prokaryotes AUG codes N–formyl methionine.
* Some times GUG also functions as start codon it codes for valine amino acid normally but when it is present at starting position it codes for methionine amino acid.
* Out of 64 codons 3–codons are stopping or nonsense or termination codon.

Nonsense codons do not specify any amino acid.

UAA (Ochre)
UAG (Amber) } Non–Sense Codons or Stop codons
UGA (Opal)

* So only 61 codons are sense codons which specify 20 amino acid.

Question-1: Genetic code was discovered by :-

(1) Gamow (2) Kornberg & Crick
(3) Khorana & Kornberg (4) Nirenberg & Mathei

Answer: (1) Nirenberg & Matthaei

Question-2: 64 Codons constitute genetic code because-

(1) There was 64 types of amino acid (2) 64 types of tRNA
(3) Genetic code is triplet (4) There are 64 enzymes

Answer: (3) Genetic code is triplet

Question-3: Genetic code determines-

(1) Structural pattern of an organism (2) Sequence of amino acid in protein chain
(3) Variation in offsprings (4) constancy of morphological trait

Answer: (2) Sequence of amino acid in protein chain

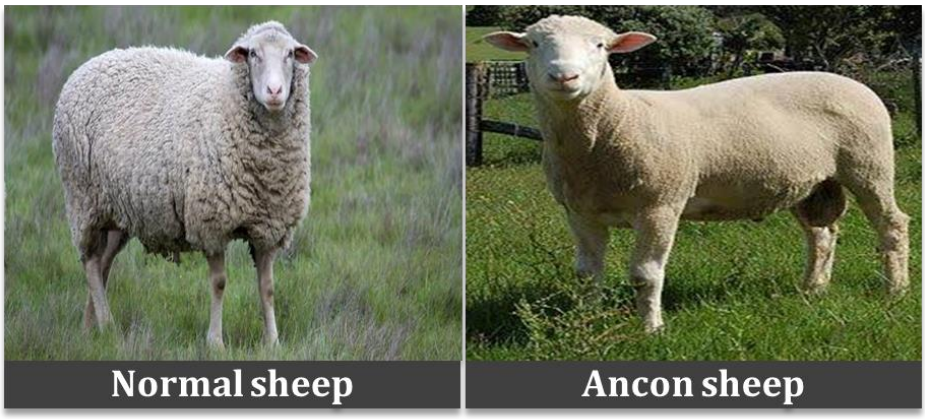
Question-4: Sometimes the starting codon is GUG in place of AUG, GUG normally stands for :-

(1) Valine (2) Glycine (3) Methionine (4) Tyrosine

Answer: (1) Valine

MUTATION

* Sudden heritable change in genetic material of an organism is called as **Mutation**.
* Mutation are source of discontinuous variation.
* Only those mutation are heritable which occur in **germinal cell** of an organism. While somatic mutations are non-heritable. **Somatic mutations are also heritable in vegetative propagated plants.**
* Mutation word was given by **Hugo De Vries**.
* De Vries studied mutations in the plant ***Oenothera lamarckiana*** (evening primrose).
* Mutation was first observed by **Seth Wright**. who observed some short-legged sheep (Ancon) variety in a population of long-legged sheep.



* **Beadle and Tatum** induced mutations in Neurospora by the help of U. V. rays or X-rays.

$$\underset{\text{Prototroph}}{\text{Normal (wild) Neurospora}} \xrightarrow{\text{U.V. rays}} \underset{\text{Auxotroph}}{\text{Mutant Neurospora}}$$

Normal-**Neurospora** can be grown in minimal medium because Neurospora can make all essential nutrients required for it. This is known as **Prototroph**.

Mutant Neurospora doesn't has capability to grow in minimal medium because due to mutation it loses those genes which codes for the enzyme that helps to prepare some special nutrients for it. They gave "one gene-one enzyme" concept. This form is known as **Auxotroph**.

* M.S. Swaminathan induced mutations in wheat by the help of γ-rays to obtain good varieties for *e.g.* Sharbati Sonora, Pusa Lerma. Swaminathan established γ garden in IARI-New Delhi (Pusa Institute).

TYPES OF MUTATION :

(I) CHROMOSOMAL MUTATION

(II) GENE MUTATIONS

(I) CHROMOSOMAL MUTATIONS :

Change in number or structure of chromosome.

Types of chromosomal mutation

(1) Heteroploidy/Genomatic mutation $\rightarrow$ change in chromosome number.

(2) Chromosomal aberration $\rightarrow$ change in structure of chromosome.

(1) HETEROPLOIDY/GENOMATIC MUTATION

* Change in number of one or few chromosomes in a set or number of entire set of chromosome.

It is of two types :

(i) Euploidy $\rightarrow$ Change in number of chromosome sets.

(ii) Aneuploidy $\rightarrow$ Change in number of chromosome in a set.

Euploidy :

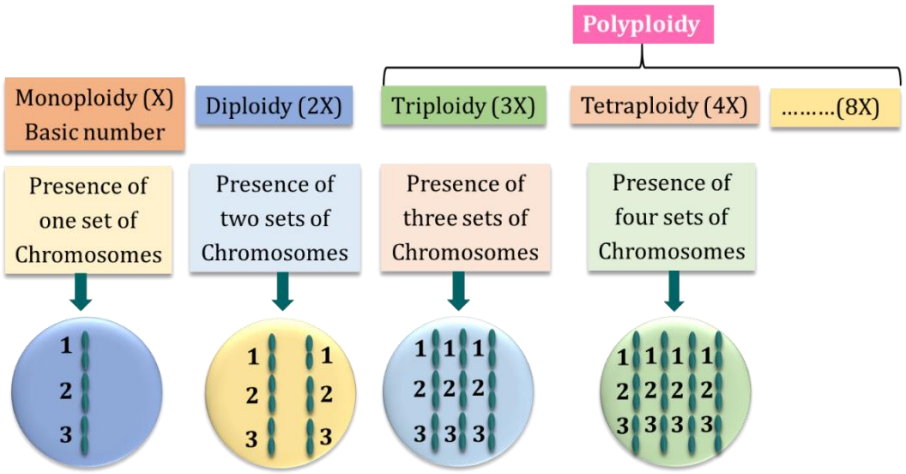
* Change in number of sets of chromosome i.e. either loss or addition of sets of chromosomes.
* Monoploidy (x) - Presence of one set of chromosomes.
* Diploidy (2x) - Presence of two sets of chromosomes.
* Polyploidy - Presence of more than two sets of chromosomes.

  It may be

  Triploidy (3x)

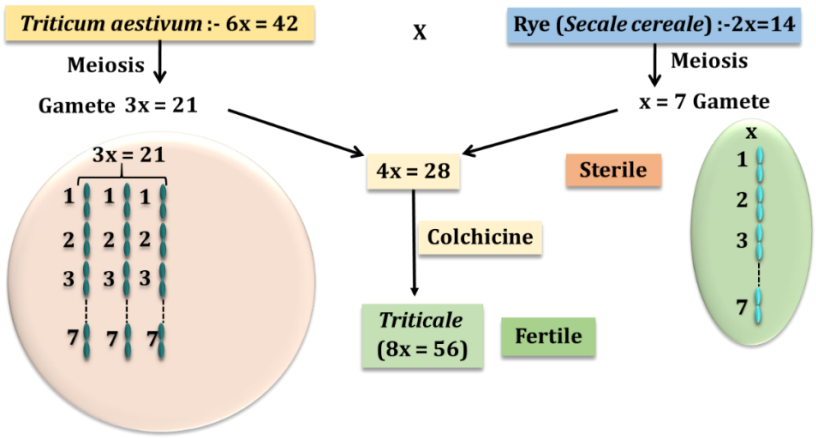
  Tetraploidy (4x)

  Pentaploidy (5x)



* Polyploid plants with even number of sets are always fertile, reproduce sexually and form seeds.
* Polyploid plants with odd number of sets are always sterile don't reproduce by sexual reproduction, They don't produce seeds but they may produce seedless fruits by parthenocarpy. ***e.g.*** Banana and seedless grapes.

Example :



Aneuploidy :

Loss or addition of chromosomes in a set of chromosomes.

Types of Aneuploidy :

1. Hypoaneuploidy (loss)

* **2n – 1 = Monosomy :-** (loss of one chromosome in one set).
    * **2n – 1 – 1 = Double monosomy :-** (loss of one chromosome from each set, but these are non homologous.)
* **2n – 2 = Nullisomy :-** (loss of two homologous chromosome)

2. Hyperaneuploidy (addition)

* **2n + 1 = Trisomy :-** addition of one chromosome in one set.
* **2n + 1 + 1 = Double Trisomy :-** addition of one chromosome in each set.
* **2n + 2 = Tetrasomy :-** addition of two chromosome in one set.
* Cause of aneuploidy is chromosomal non-disjunction means chromosomes fail to separate during meiosis.
* Chances of aneuploidy are more in higher age female due to less activity of oocyte, so chances of syndrome increase in children who are born from higher age female.

(2) CHROMOSOMAL ABERRATIONS :

Change in structure of chromosome.

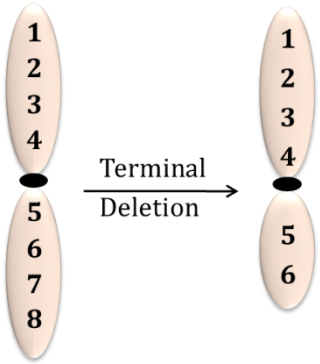
(i) Deletion :

Loss of a part or segment of chromosome which leads to loss of some genes is called as deletion.

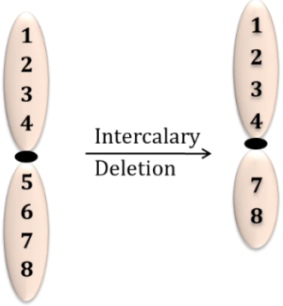
It is of 2 types :-

(a) Terminal deletion - Loss of chromosomal segment from one or both ends.

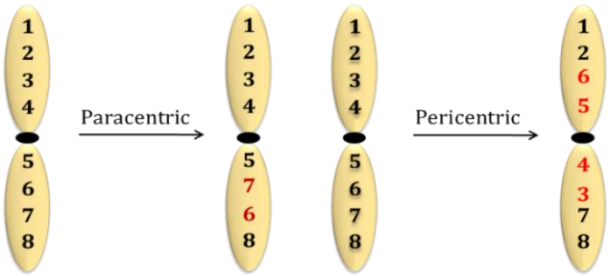
***e.g.* The cry -du-chat syndrome is an example of terminal deletion in short arm of 5th chromosome.**



(b) Intercalary deletion - Loss of chromosomal part between the ends.



(ii) **Inversion :-** Breakage of chromosomal segment but reunion on same chromosome in reverse orders. It leads to change in distance between genes on chromosome or sequence of genes on chromosome so crossing over is affected.



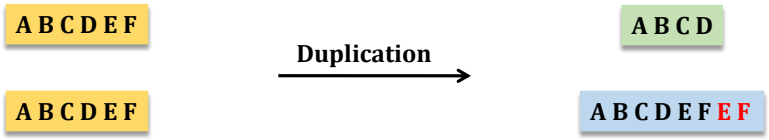
It is of 2 types :-

(a) Paracentric - If inversion occurs only in one arm and inverted segment does not include centromere.

(b) Pericentric - In this type of inversion inverted segment include centromere.

(iii) **Duplication :-** Occurrence of a chromosomal segment twice on a chromosome.

Example : In drosophila **"Bar eye character"** is observed due to duplication in X-chromosome. Bar eye is a character where eyes are narrower as compared to normal eye shape.

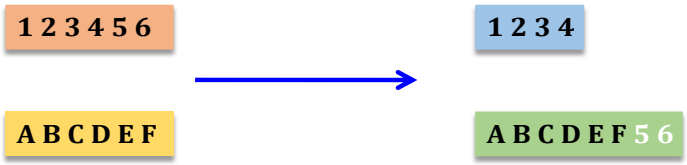


Homologous chromosomes

(iv) **Translocation : -** In this, a part of the chromosome is broken and may be joined with non-homologous chromosome. This is also known as Illegitimate crossing over (illegeal crossing over)

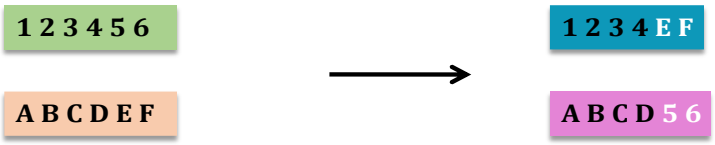
Types of translocation –

(A) **Simple Translocation**→ When a chromosomal segment breaks and attached to the terminal end of a non- homologous chromosome.



Non - Homologous chromosomes

(B) **Reciprocal Translocation**→ Exchange of segments between two non-homologous chromosome.



Non - Homologous chromosomes

e.g. Chronic Myeloid Leukemia [CML] is a type of blood cancer. This disease is a result of reciprocal translocation between 22 and 9 chromosome.

Note : If exchange of segments takes place in between homologous chromosomes then it is **called crossing over**.

(II) GENE MUTATION OR POINT MUTATION

Two types :-

(1) Substitution

(2) Frame shift mutation.

(1) SUBSTITUTION :

Replacement of one nitrogenous base by another nitrogenous base is called as substitution.

* It causes change in one codon in genetic code which leads to change in one amino acid in structure of protein. ***e.g.*** Sickle cell anaemia
* Change may not occur some time because for one amino acid more than one type of codons are present.

Substitution is of two types :

1. Transition :

Replacement of one purine by another purine or replacement of pyrimidine by another pyrimidine.

2. Transversion :

Replacement of purine by pyrimidine or pyrimidine by purine is called transversion.

(2) FRAME SHIFT MUTATION/GIBBERISH MUTATION :

Loss or addition of one or rarely more than one nitrogenous bases in structure of DNA. Frame shift mutation is of two types

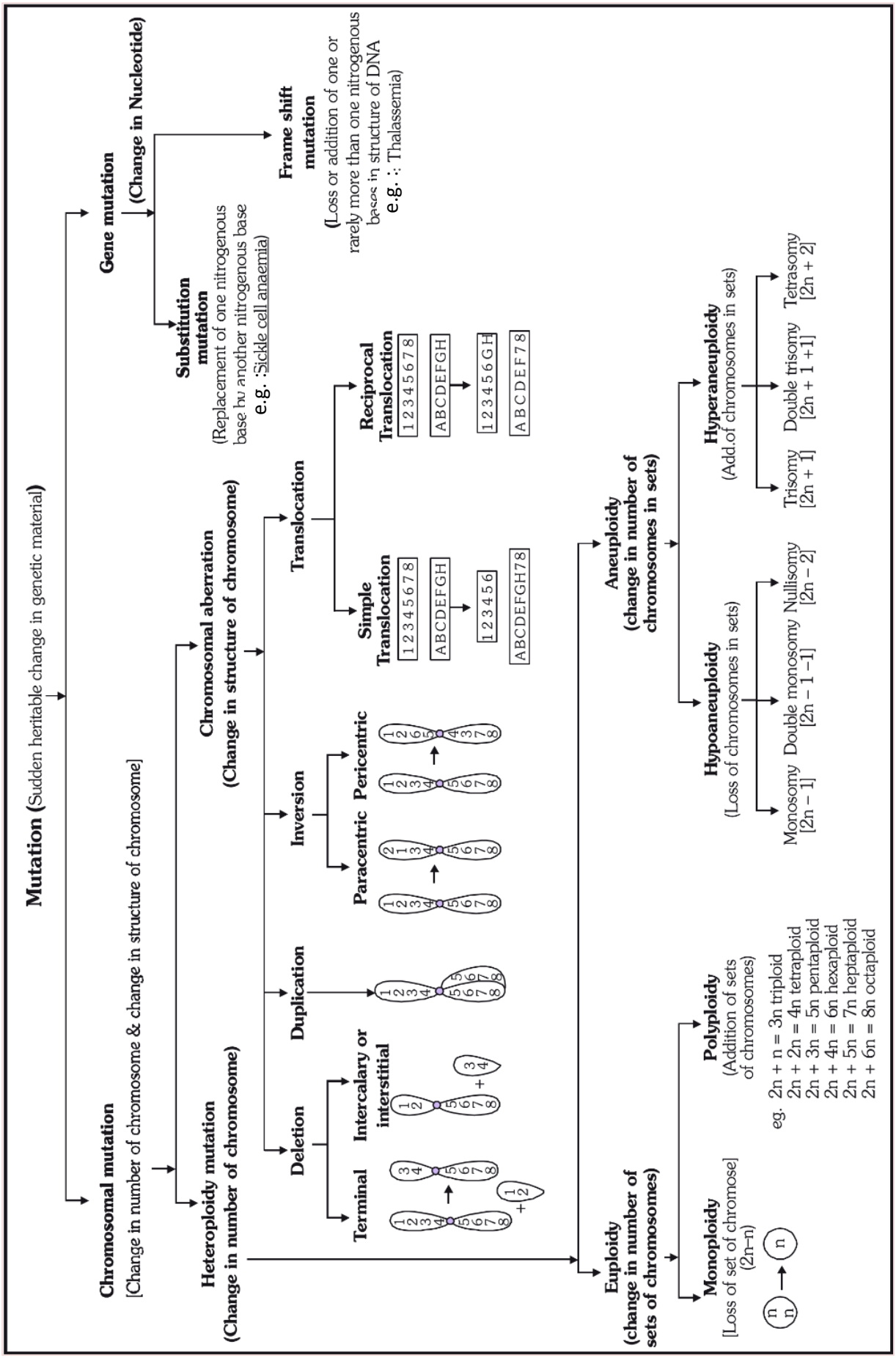
1. Addition :- Addition of one or rarely more than one nitrogenous bases in structure of DNA.

2. Deletion :- Loss of one or rarely more than one nitrogenous bases in structure of DNA.

Due to frame shift mutation complete reading of genetic code is changed. It leads to change in all amino acids in structure of protein so a new protein is formed which is completely different from previous protein.

* So, frame shift mutations are more harmful as compared to substitution.

e.g. : Thalassemia (lethal genetic disorder)


Mutation (Sudden heritable change in genetic material)
Gene mutation
(Change in Nucleotide)
Substitution mutation
(Replacement of one nitrogenous base by another nitrogenous base
e.g. :Sickle cell anaemia)
Frame shift mutation
(Loss or addition of one or rarely more than one nitrogenous bases in structure of DNA
e.g. :: Thalassemia)
Chromosomal mutation
[Change in number of chromosome & change in structure of chromosome]
Heteroploidy mutation
(Change in number of chromosome)
Chromosomal aberration
(Change in structure of chromosome)
Deletion
Terminal
Intercalary or interstitial
Duplication
Inversion
Paracentric
Pericentric
Translocation
Simple Translocation
Reciprocal Translocation
1 2 3 4 5 6 7 8
A B C D E F G H
1 2 3 4 5 6
A B C D E F G H 7 8
1 2 3 4 5 6 G H
A B C D E F 7 8
Euploidy
(change in number of sets of chromosomes)
Monoploidy
[Loss of set of chromose]
(2n–n)
Polyploidy
(Addition of sets of chromosomes)
eg. 2n + n = 3n triploid
2n + 2n = 4n tetraploid
2n + 3n = 5n pentaploid
2n + 4n = 6n hexaploid
2n + 5n = 7n heptaploid
2n + 6n = 8n octaploid
Aneuploidy
(change in number of chromosomes in sets)
Hypoaneuploidy
(Loss of chromosomes in sets)
Monosomy [2n – 1]
Double monosomy [2n – 1 –1]
Nullisomy [2n – 2]
Hyperaneuploidy
(Add.of chromosomes in sets)
Trisomy [2n + 1]
Double trisomy [2n + 1 +1]
Tetrasomy [2n + 2]

MUTAGENS :

* Mutagens are those substances which cause mutations.

Non ionising :- U. V. rays.

* Mostly mutations are harmful.
* Sometimes they are lethal which leads to death of organisms.
* But sometimes they are beneficial which are used to obtain good varieties of plants and animals. It is called as

Mutation Breeding.

* Mostly mutations are recessive and they never eliminate from a population.

Forward and Backward Mutation :

* Wild gene $\underset{\text{Backward}}{\overset{\text{Forward}}{\rightleftharpoons}}$ Mutant gene

Muton (unit of mutation):

* Smallest part of DNA which undergoes mutation.
* It is one nucleotide.

Mis-sense mutation :-

* When a nucleotide change in genetic code causes the change of one amino acid of a polypeptide chain it is called mis-sense mutation.

Non-sense mutation :-

* When a nucleotide change in one codon causes termination of polypeptide synthesis by producing non-sense codon.

Same sense codon :-

* A change in one nucleotide in a codon does not change amino acid in polypeptide chain, because both codons code same amino acid.

Question-1: Mutation is -

(1) An abrupt or discontinuous change which is inherited

(2) A factor for plant growth

(3) A change which affects parents only and is never inherited

(4) A change which affects the offspring of F_2 generation

Answer: (1) An abrupt or discontinuous change which is inherited

Question-2: The change of chromosomal parts between non homologous pairs of chromosome -

(1) Crossing over/Transduction (2) Translocation

(3) Inversion (4) Transition

Answer: (2) Translocation

Question-3: Genetic mutations occur in eukaryotes -

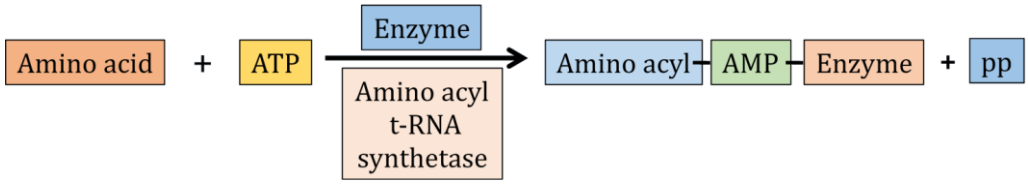
(1) Protein (2) RNA (3) DNA (4) RNA & DNA

Answer: (4) RNA & DNA

TRANSLATION

(1) ACTIVATION OF AMINO ACID :–

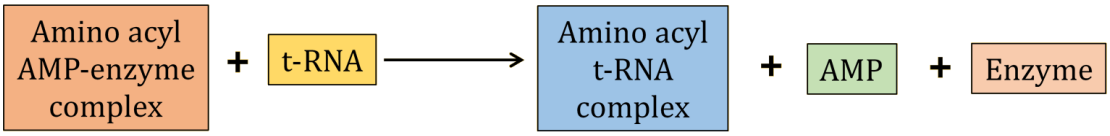
* 20 types of amino acids participate in protein synthesis.
* Amino acid reacts with ATP to form **''Amino acyl AMP enzyme complex''**, which is also known as **'Activated Amino acid'**.



* This reaction is catalyzed by a specific 'Amino acyl tRNA synthetase' enzyme.
* There is a separate 'Amino acyl tRNA synthetase' enzyme for each kind of amino acid.

(2) CHARGING OF T–RNA (LOADING OF T-RNA) :–

* Specific activated amino acid is recognised by its specific tRNA.
* Now amino acid attaches to the 'Amino acid attachment site' of its specific tRNA and AMP and enzyme are separated from it.



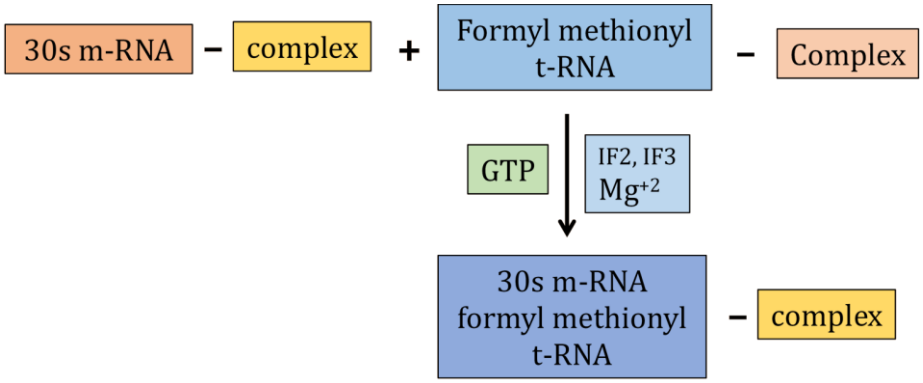
* Amino acyl tRNA complex is also called 'Charged tRNA'.
* Now Amino acyl tRNA moves to the ribosome for protein synthesis.

(3) TRANSLATION :– 3 steps –

(A) INITIATION OF POLYPEPTIDE CHAIN :–

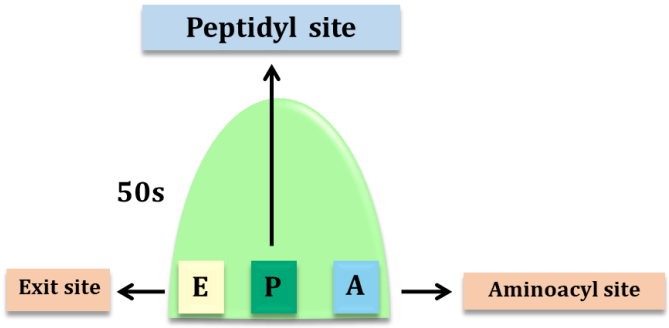
* In this step 30s and 50s sub units of ribosome, GTP, Mg^{+2}, charged tRNA, mRNA and some initiation factors are required.
* In prokaryotes there are three initiation factors present – **IF1, IF2, IF3.**
* Initiation factors are specific protein.
* GTP and initiation factors promote the initiation process.
* In prokaryotes with the help of **''S D sequence'' (Shine–Delgarno sequence)** mRNA recognises the smaller subunit of ribosome. A sequence of 8 N_2 base is present before the 4-12 N_2 base of initiation codon on mRNA, called "**SD sequence**". In Smaller subunit of ribosome, a complementary sequence of "SD sequence" is present on 16s rRNA, which is called "**Anti Shine-Delgarno sequence**" (ASD sequence)
* With the help of 'SD' and 'ASD sequence' mRNA recognises the smaller sub unit of ribosome.
* While in eukaryotes, smaller subunit of ribosome is recognised by **"7mGcap"**.
* In eukaryotes, 18s rRNA of smaller sub unit has a complementary sequence of **"7mGcap"**.

* This ''30s mRNA – complex'' reacts with 'Formyl methionyl tRNA – complex' and 30s mRNA – formyl methionyl tRNA – complex'' is formed. This tRNA attaches with codon part of mRNA. A GTP molecule is required.

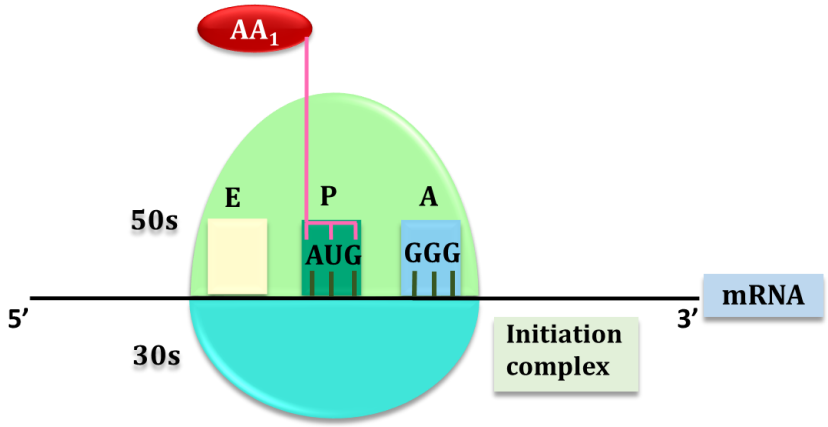


* Now larger sub unit of ribosome (50s subunit) joins this complex.
* In larger subunit of ribosome there are three sites for tRNA –

'P' site = Peptidyl site.
'A' site = Amino acyl site.
'E' site = Exit site



* Starting codon of mRNA is near to 'P' site of ribosome, so tRNA with formyl methionine amino acid first attaches to **'P' site** of ribosome and next codon of mRNA is near to 'A' site of ribosome. So next new tRNA with new amino acid always attach at **'A' site** of ribosome but in initiation step **'A' site** is empty.



Question-1: Transfer of genetic information from a polymer of nucleotides to a polymer of amino acids is –

(1) Replication (2) Transcription

(3) Translation (4) Reverse transcription

Answer: (3) Translation

Question-2: Site of tRNA, where amino acid is attached?

(1) 3' end (2) 5' end (3) Anticodon (4) DHU loop

Answer: (1) 3' end

Question-3: How many types of amino acids participate in protein synthesis :-

(1) 20 type (2) 18 type (3) 22type (4) 32 type

Answer: (1) 20 type

Question-4: Which is also known as 'Activated Amino acid'.

(1) NADPH complex (2) Amino acyl AMP enzyme complex

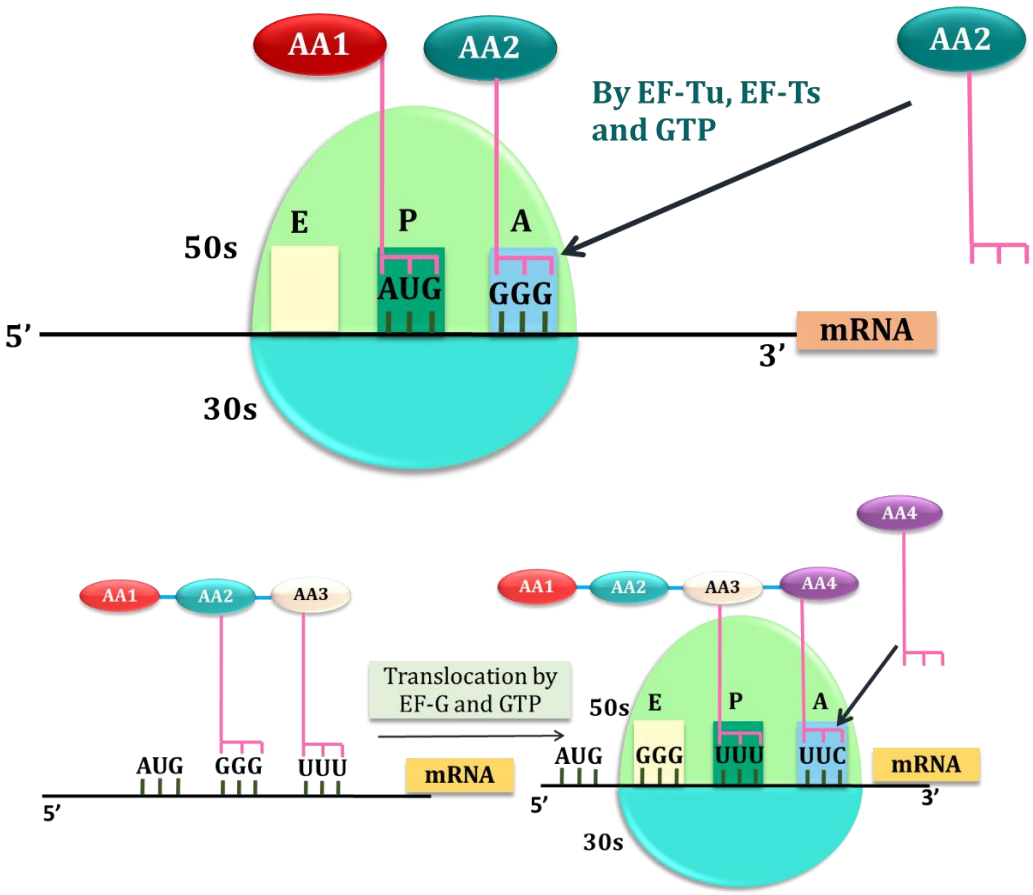
(3) Oxide reductase complex (4) Charged tRNA

Answer: (2) Amino acyl AMP enzyme complex

TRANSLATION

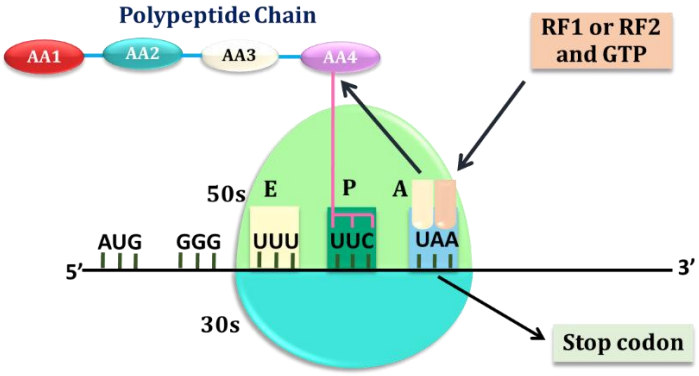
(B) CHAIN – ELONGATION :-

* New tRNA with new amino acid is attaches at **'A' site** of ribosome.
* The link between amino acid of **'P' site** of tRNA is broken and tRNA of **'P' site** is discharged so –COOH of **'P' site** amino acid becomes free.
* Now peptide bond takes place between **– COOH group** of **P site** amino acid and **$-NH_2$** group of **A site** amino acid.
* 23s rRNA induces the formation of peptide bond. This rRNA acts as an enzyme so it is called **''Ribozyme''**.
* After formation of peptide bond tRNA of **P site** released from ribosome via **E site** and dipeptide attaches with **A site**.
* Now tRNA of **A site** is transferred to **P site** and hence **A site** becomes empty.
* Now ribosome slides over mRNA strand in **5' → 3'** direction. Due to sliding of ribosome on mRNA, new codon of mRNA continuously available at **A site** of ribosome and according to new codon of mRNA new amino acid attaches in polypeptide chain.
* Translocase enzyme is helpful in movement of ribosome (translocation). GTP provides energy for sliding of ribosome.
* In elongation process some protein factors are also helpful, which are known as **'Elongation factors'**.
* In prokaryotes three 'Elongation factors' are present – **EF–Tu, EF–Ts, EF–G**.



(C) CHAIN – TERMINATION :–

* Due to sliding of ribsome over mRNA when any Nonsense codon (UAA, UAG, UGA) available at **A site** of ribosome, then polypeptide chain terminates.
* The linkage between the last tRNA and the polypeptide chain is broken by three **release factor** called **RF1, RF2, RF3** with the help of **GTP**.



* An mRNA also have some additional sequences that are not translated and are referred as untranslated regions (UTR). The UTRs are present at both 5'end (before start codon) and at 3'end (after stop codon).
* The UTR(untranslated regions) present on mRNA are required for efficient translation process (by recognising the smaller subunit of ribosome by mRNA.)

Question-1: Translation refers to the process of -

(1) Polymerisation of nitrogen bases (2) Polymerisation of nucleotides

(3) Polymerisation of nucleosides (4) Polymerisation of amino acids

Answer: (4) Polymerisation of amino acids

Question-2: Amino acid sequence, in protein synthesis is decided by the sequence of -

(1) tRNA (2) mRNA (3) cDNA (4) rRNA

Answer: (4) mRNA

Question-3: Which of the following rRNA acts as structural RNA as well as ribozyme in bacteria -

(1) 23S rRNA (2) 5.8S rRNA (3) 5S rRNA (4) 18S rRNA

Answer: (1) 23S rRNA

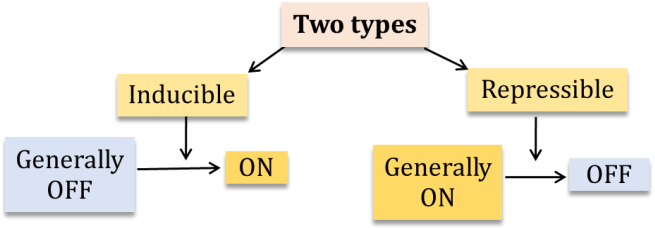
REGULATION OF GENE

CONSTITUTIVE GENES (HOUSE-KEEPING GENES) :–

These genes are expressed constantly, because their products are constant needed for cellular activity. e.g. genes for glycolysis, gene of ATPase enzyme.

NON-CONSTITUTIVE GENES (SMART GENE OR LUXURY GENE) :–

These genes remain silent and are expressed only when the gene product is needed. They are switched 'on' or 'off' according to the requirement of cellular activities. Non-constitutive genes are of two types; **inducible** and **repressible**. The inducible genes are switched on in presence of a chemical substance called **inducer**, required for the functioning of gene activity. The repressible genes continue to express themselves till a chemical, often an end product of the metabolism inhibits or represses their activity. Such type of inhibition is called feed back inhibition or feed back repression.



- The mechanism which stimulates the expression of certain genes and inhibits that of others is called **regulation of gene expression**.
- It is possible only if the organism has a mechanism of regulating gene activity by allowing some to function and others to restrain their activity through switching on and switching off system. This means, the genes are turned 'on' or 'off' as per requirement.
- A set of genes is 'switched on' when enzymes are required to metabolise a new substrate. The enzymes produced by these genes metabolise the substrate.

- The molecules of metabolite that come to switch on of the genes are termed as inducers and the phenomenon is called **induction**.
- Similarly, certain genes which are in their 'switch on' state, continue to synthesise a metabolite till the later is produced in amount more than required or else, it is supplied to the cell from outside. In other words, certain genes continue to express themselves till the end product of inhibits or represses their expression. Inhibition by an end product is known as 'feedback repression'.
- Regulation of gene expression refers to a very broad term that may occur at various levels. Considering that gene expression results in the formation of a polypeptide, it can be regulated at several levels.
- In eukaryotes, the regulation could be exerted at
  (i) transcriptional level (formation of primary transcript),
  (ii) processing level (regulation of splicing),
  (iii) transport of mRNA from nucleus to the cytoplasm
  (iv) translational level.

Question-1: Which is the primary step for regulation of gene expression?

(1) Transport of m-RNA from nucleus to the cytoplasm
(2) Translational level
(3) Processing level
(4) Transcriptional level

Answer: (4) Transcriptional level

Explanation:

In eukaryotes, the regulation could be exerted at
(1) transcriptional level (formation of primary transcript),
(2) processing level (regulation of splicing),
(3) transport of mRNA from nucleus to the cytoplasm
(4) translational level.

Question-2: Which of the following genes are expressed constantly?

(1) Constitutive genes (2) Non-constitutive genes
(3) Smart genes (4) Luxury genes

Answer: (1) Constitutive genes

Question-3: Genes of glycolysis is -

(1) Constitutive genes (2) Non-constitutive genes
(3) Smart genes (4) Luxury genes

Answer: (1) Constitutive genes

Explanation:

These genes are expressed constantly, because their products are constant needed for cellular activity. e.g. genes for glycolysis, gene of ATPase enzyme.

OPERON CONCEPT

- In 1961, two French microbiologist ***Francis Jacob*** and ***Jacques Monod*** at the Pasteur Institute in Paris, proposed a mechanism called operon model for the regulation of gene action in ***E. coli.***
- An operon is a part of genetic material or DNA, which acts as a single regulated unit having one or more structural genes-an operator gene, a promoter gene, a regulator gene.
- Operons are of two types (i) inducible (ii) repressible.

1. INDUCIBLE SYSTEM (LAC OPERON OF *E. COLI*)

An inducible operon system normally remains in switched off condition and begins to work only when the substance to be metabolised by it is present in the cell. Inducible operon system generally occurs in catabolic pathways. ***e.g.*** Lac operon of ***E. coli.***

Active repressor + Inducer = Inactive repressor

An inducible operon system consists of four types of genes

(i) **Structural genes -** These genes synthesise mRNAs, which in turn synthesise polypeptide or enzyme over the ribosomes. An operon may have one or more structural genes. Each structural gene of an operon is called cistron. The lac operon (lactose operon) of ***Escherichia coli*** contains three structural genes (Z, Y and A). These genes occur adjacent to each other and thus are linked. They transcribe a polycistronic mRNA molecule (a single stretch of mRNA covering all the three genes), that helps in the synthesis of three enzymes-β galactosidase (breaks lactose into glucose and galactose), lactose permease (helps in entry of β galactosidase (lactose) in cell from outside) and transacetylase (transfers an acetyl group from acetyl Co A to b galactosidase).

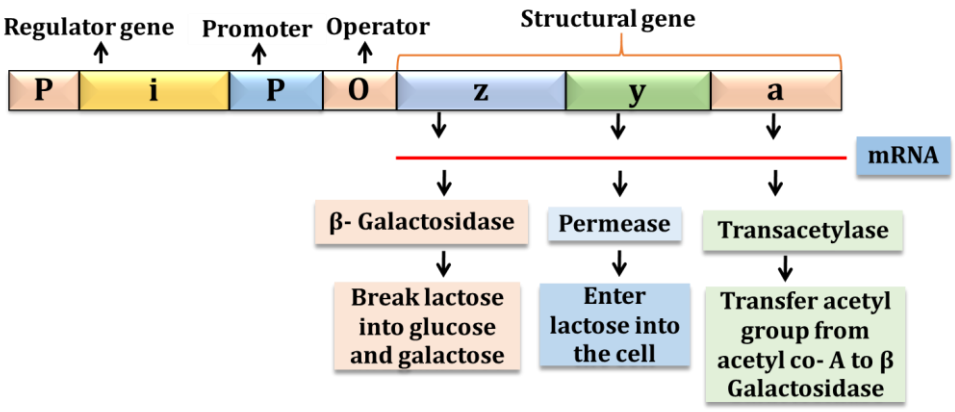
The lac operon consists of one regulatory gene (the i gene – here the term i does not refer to inducer, rather it is derived from the word inhibitor) and three structural genes (z, y, and a). The i gene codes for the repressor of the lac operon. The z gene codes for β - galactosidase (b-gal), which is primarily responsible for the hydrolysis of the disaccharide, lactose into its monomeric units, galactose and glucose. The y gene codes for permease, which increases permeability of the cell to β-galactosidase.

The a gene encodes a transacetylase. Hence, all the three gene products in lac operon are required for metabolism of lactose. In most other operons as well, the genes present in the operon are needed together to function in the same or related metabolic pathway.

(ii) **Operator gene -** It lies adjacent to the structural genes and directly controls the synthesis of mRNA over the structural genes. It is switched off by the presence of a repressor. An inducer can take away the repressor and switch on the gene that directs the structural genes to transcribe.

(iii) **Promoter gene -** This gene is the site for initial binding of RNA polymerase. When the operator gene is turned on, the enzyme RNA polymerase moves over it and reaches the structural genes to perform transcription.

(iv) **Regulator gene -** It produces a repressor that binds to operator gene and stops the working of the operator gene.



Repressor - It is a protein, produced by the regulator gene. It binds to the operator gene so that the transcription of structural gene stops. Repressor has two binding site (1) operator gene (2) effective molecule (inducer/corepressor)

Inducer - It is a chemical (substrate, hormone or some other metabolite) which after coming in contact with the repressor, forms an inducer repressor complex. This complex cannot bind with the operator gene, which is thus switched on. The free operator gene allows the structural gene to transcribe mRNA to synthesise the enzymes.

u

cer for lac operon of ***Escherichia coli*** is lactose (in fact allolactose an isomer of lactose). When the sugar lactose is added to the culture of ***E. coli***, a few molecules of lactose gets into the bacterial cells by the action of the enzyme permease, a small amount of this enzyme is present in the cell even when the operon is not working. These few lactose molecules are then converted into an active form which acts as an inducer and binds to the repressor protein. The inducer repressor complex fails to join with the operator, which is turned on. The three genes are expressed as three enzymes to metabolise lactose. Allolactose is real inducer of lac operon.

Special points of lac operon

1. Lac :- lactose.
2. *i gene* :- Constitutive genes.
3. *z, y and a gene* :- non -constitutive genes.
4. Catabolic pathway.
5. Inducible operon (off → on).
6. Inducer :- lactose.
7. Real inducer :- allolactose.
8. A very low level of expression of lac operon has to be present in the cell all the time, otherwise lactose cannot enter the cells.

NCERT Insider :-

Regulation of *lac* operon by repressor is referred to as negative regulation. *Lac* operon is under control of positive regulation as well, but it is beyond the scope of discussion at this level.

Question-1: Which one of following is acts as an inducer in lac operons?

(1) Lactose (2) Arabinose
(3) Tryptophan (4) Phenylalanine

Answer: (1) Lactose

Question-2: Who explained the operon model for the first time?

(1) Francis Jacob (2) Jacques Monod
(3) Francis Jacob and Jacques Monod (4) Beadle & Tatum

Answer: (3) Francis Jacob and Jacques Monod

Question-3: Which is incorrect :-

(1) i-gene codes for the repressor of lac operon
(2) z-gene codes for the beta-galactosidase
(3) y-gene codes for transacetylase
(4) Three gene products are required for metabolism of lactose

Answer: (3) y-gene codes for transacetylase

Question-4: Regulation of lac operon by repressor is referred to as :-

(1) Positive regulation (2) Negative regulation
(3) Both (1) and (2) (4) No regulation

Answer: (2) Negative regulation

Question-5: What does "lac" refer to, in what we call the lac operon?

(1) Lactose (2) Lactase (3) Lac insect (4) Lactification

Answer: (1) Lactose

Question-6: Which of the following is not produced by *E.Coli* in the lactose operon?

(1) β – galactosidase (2) Permease
(3) Lactose dehydrogenase (4) Transacetylase

Answer: (3) Lactose dehydrogenase

HUMAN GENOME PROJECT

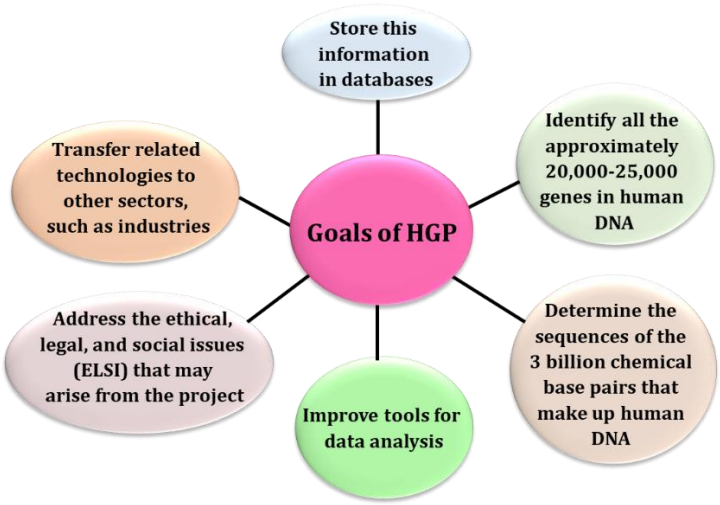
Genetic make-up of an organism or an individual lies in the DNA sequences. If two individuals differ, then their DNA sequences should also be different, at least at some places. These assumptions led to the quest of finding out the complete DNA sequence of human genome. With the establishment of genetic engineering techniques where it was possible to isolate and clone any piece of DNA and availability of simple and fast techniques for determining DNA sequences, a very ambitious project of sequencing human genome was launched in the year 1990.

Human Genome Project (HGP) was called a mega project. You can imagine the magnitude and the requirements for the project if we simply define the aims of the project as follows :

Human genome is said to have approximately 3×10^9 bp, and if the cost of sequencing required is US $ 3 per bp (the estimated cost in the beginning), the total estimated cost of the project would be approximately 9 billion US dollars. Further, if the obtained sequences were to be stored in typed form in books, and if each page of the book contained 1000 letters and each book contained 1000 pages, then 3300 such books would be required to store the information of DNA sequence from a single human cell. HGP was closely associated with the rapid development of a new area in biology called as **Bioinformatics**.

Goals of HGP

Some of the important goals of HGP are as follows :


Goals of HGP
Store this information in databases
Identify all the approximately 20,000-25,000 genes in human DNA
Determine the sequences of the 3 billion chemical base pairs that make up human DNA
Improve tools for data analysis
Address the ethical, legal, and social issues (ELSI) that may arise from the project
Transfer related technologies to other sectors, such as industries

The project was completed in 2003. Knowledge about the effects of DNA variations among individuals can lead to revolutionary new ways to diagnose, treat and someday prevent the thousands of disorders that affect human beings. Besides providing clues to understanding human biology, learning about non-human organisms, DNA sequences can lead to an understanding of their natural capabilities that can be applied toward solving challenges in health care, agriculture, energy production, environmental remediation. Many non-human model organisms, such as bacteria, yeast, *Caenorhabditis elegans* (a freeliving non-pathogenic nematode), Drosophila (the fruit fly), plants (rice and Arabidopsis), etc., have also been sequenced.

METHODOLOGIES :-

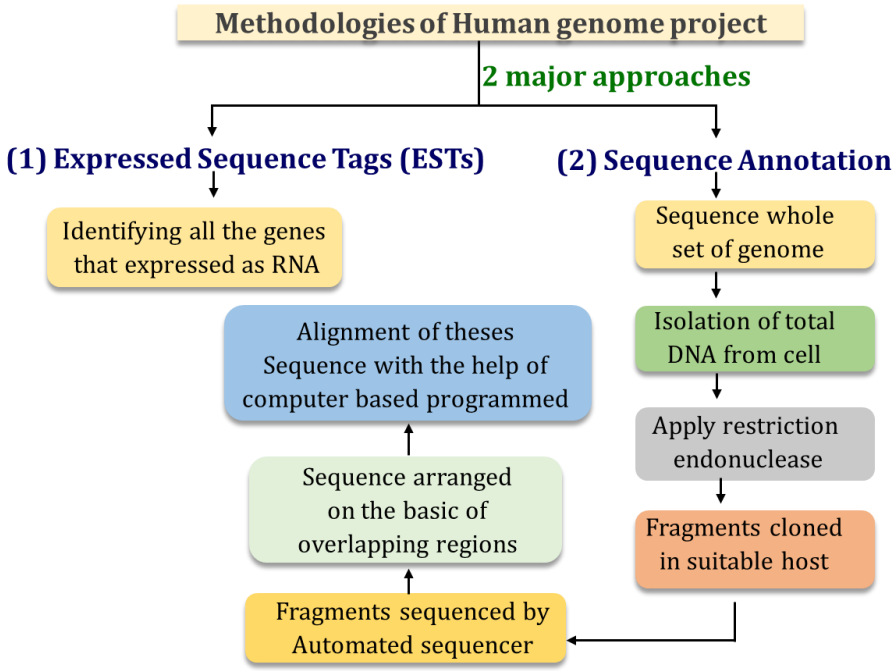
The methods involved two major approaches.

EXPRESSED SEQUENCE TAGS (ESTS) :-

Identifying all the genes that expressed as RNA.

SEQUENCE ANNOTATION :-

The blind approach of simply sequencing the whole set of genome that contained all the coding and non-coding sequence, and later assigning different regions in the sequence with functions. For sequencing, the total DNA from a cell is isolated and converted into random fragments of relatively smaller sizes (recall DNA is a very long polymer, and there are technical limitations in sequencing very long pieces of DNA) and cloned in suitable host using specialised vectors. The cloning resulted into amplification of each piece of DNA fragment so, that is subsequently could be sequenced with ease. The commonly used hosts were bacteria and yeast, and the vectors were called as **BAC (bacterial artificial chromosomes)**, and **YAC** (yeast artificial chromosomes).


Methodologies of Human genome project
2 major approaches
(1) Expressed Sequence Tags (ESTs)
(2) Sequence Annotation
Identifying all the genes that expressed as RNA
Sequence whole set of genome
Isolation of total DNA from cell
Apply restriction endonuclease
Fragments cloned in suitable host
Fragments sequenced by Automated sequencer
Sequence arranged on the basic of overlapping regions
Alignment of theses Sequence with the help of computer based programmed

The fragments were sequenced using automated DNA sequencers that worked on the principle of a method developed by **Frederick Sanger**. (Remember, Sanger is also credited for developing method for determination of amino acid sequences in proteins). These sequences were then arranged based on **some overlapping regions** present in them. This required generation of overlapping fragments for sequencing. Alignment of these sequences was humanly not possible. Therefore, specialised computer-based programmes were developed. These sequences were subsequently annotated and were assigned to each chromosome. The sequence of chromosome I was completed only in May 2006 (this was the last of the 24 human chromosomes - 22 autosomes and X and Y- to be sequenced). Another challenging task was assigning the genetic and physical maps on the genome. This was generated using information on polymorphism of restriction endonuclease recognition sites, and some repetitive DNA sequences known as microsatellites.

SALIENT FEATURES OF HUMAN GENOME :–

Some of the salient observations drawn from human genome project are as follows :

(i) The human genome contains 3164.7 million nucleotide bases.

(ii) The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.

(iii) The total number of genes is estimated at 30,000-much lower than previous estimates of 80,000 to 1,40,000 genes. Almost all (99.9 per cent) nucleotide bases are exactly the same in all people.

(iv) The functions are unknown for over 50 per cent of discovered genes.

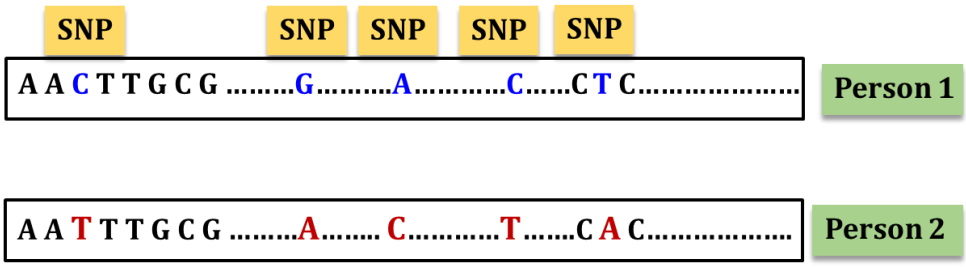
(v) Less than 2 per cent of the genome codes for proteins.

(vi) Repeated sequences make up very large portion of the human genome.

(vii) Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times. They are thought to have no direct coding functions, but they shed light on chromosome structure, dynamics and evolution.

(viii) Chromosome 1 has most genes (2968) and the Y has the fewest (231).

(ix) Scientists have identified about 1.4 million locations where single-base DNA differences **(SNPs- single nucleotide polymorphism, pronounced as 'snips')** occur in humans, This information promises to revolutionise the processes of finding chromosomal locations for disease-associated sequences and tracing human history.



Organisms	Base pair	Gene No.
Bateriophage	10,000	------/-----
Lily	106 Billion B.P.	------
E.coli	4.7 million B.P.	4,000
S. cerevisiae	12 Million B.P.	6,000
D. melangaster	180 Million B.P.	13,000
Caenorhabditis elegans	97 Million B.P.	18,000
Human	3 Billion B.P.	30,000

(a) First prokaryotes in which complete genome was sequenced is ***Haemophilus influenzae***.

(b) First Eukaryote in which complete genome was sequenced is ***Saccharomyces cerviceae (Yeast)***.

(c) First plant in which complete genome was sequenced is ***Arabidopsis thaliana (Small mustard plant)***.

(d) First animal in which complete genome was sequenced is ***Caenorhabditis elegans (Nematode)***.

β– globin and insulin gene are less than 10 kilo base pair Duchenne muscular Dystrophy gene is made up of 2400 kilo base pair. (Longest gene)

Question-1: Which of the following is not associated with HGP?

(1) Bioinformatics (2) Cloning vectors BAC and YAC

(3) Automated DNA sequencers (4) VNTRs

Answer: (4) VNTRs

Question-2: First Prokaryote, in which complete genome was sequenced is?

(1) Haemophilus influenzae (2) T4 phage

(3) M13 phage (4) Lambda phage

Answer: (1) Haemophilus influenzae

Question-3: How many genes are present on first chromosome?

(1) 2968 (2) 2986 (3) 2868 (4) 2886

Answer: (3) 2968

Question-4: In which year human genome project completed?

(1) 1990 (2) 1992 (3) 1994 (4) 2003

Answer: (4) 2003

Question-5: The largest gene in human is :-

(1) Dystrophin gene (2) Insulin gene (3) β – globin gene (4) Cry gene

Answer: (1) Dystrophin gene

DNA FINGER PRINTING / DNA TYPING / DNA PROFILING/ DNA TEST

- It is a technique to identify a person on the basis of his/her DNA specificity.
- This technique was invented by sir **Alec Jeffery (1984)**.
- In India DNA Finger printing has been started by **Dr. V.K. Kashyap & Dr. Lal Ji Singh**.
- DNA of human is almost the same for all individuals but very small amount that differs from person to person that forensic scientists analyze to identify people.
- These differences are called Polymorphism (many forms) and are the key of DNA typing. Polymorphisms are most useful to forensic scientist. It is consist of variation in the length of DNA at specific loci is called Restricted fragment. It is most important segment for DNA test made up of short repetitive nucleotide sequences, these are called VNTRs (variable number of tandem repeat).

- VNTR's also called minisatellites were discovered by **Alec Jeffery**. Restricted fragment consist of hypervariable repeat region of DNA having a basic repeat sequence of 11-60 bp and flanked on both sites by restriction site.
- The number and position of mini satellites or VNTR in restriction fragment is different for each DNA and length of restricted fragment is depend on number of VNTR.
- Therefore, when the genome of two people are cut using the same restriction enzyme the length of fragments obtained is different for both the people.
- These variations in length of restricted fragment is called RFLP or **Restriction fragment length polymorphism.**
- Restriction Fragment Length Polymorphism distributed throughout human genomes are useful for DNA Finger printing.
- DNA Fingerprint can be prepared from extremely minute amount of blood, semen, hair bulb or any other cell of the body.
  DNA content of 1 - Microgram is sufficient.

TECHNIQUE OF DNA FINGER PRINTING INVOLVES THE FOLLOWING MAJOR STEPS.

1. EXTRACTION :-

DNA extracted from the cell by cell lysis. If the content of DNA is limited then DNA can be amplified by Polymerase chain reaction (PCR). This process is amplification.

2. RESTRICTION ENZYME DIGESTION :-

Restriction enzyme cuts DNA at specific 4 or 6 base pair sequences called restriction site.
Hae III (*Haemophilus aegyptius*) is most commonly used enzyme. It cuts the DNA, everywhere the bases are arranged in the sequence GGCC. These restricted fragments transferred to Agarose Polymer gel.

3. GEL ELECTROPHORESIS :-

- Gel electrophoresis is a method that separates macromolecules-either nucleic acid or proteins-on the basis of size, electric charge.
- Gel electrophoresis refers to the technique in which molecules are forced across a span of gel, motivated by an electrical current. Activated electrodes at either end of the gel provides the driving force. A molecule's properties determine, how rapidly an electric field can move the molecule through a gelatinous medium.
- Now a days the most commonly used matrix is agarose which is a natural polymer extracted from sea weeds. The DNA fragments separate (resolve) according to their size through sieving effect provided by the agarose gel.
- Many important biological molecules such as amino acids, peptides, proteins, nucleotides, and nucleic acids possession is able groups and, therefore, at any given pH, exist in solution as

electrically charged species either as cation (+) or anions (–). Depending on the nature of the net charge, the charged particles will migrate either to the cathode or to the anode.

- By the gel electrophoresis these restricted fragments move towards the positive electrode (anode) because DNA has negative electric charge (PO_4^{-3}).
- Smaller Fragments more move towards the positive pole due to less molecular weight. So after the gel electrophoresis DNA fragment arranged according to molecular weight.
- These separated fragments can be visualized by staining them with a dye that fluoresces ultraviolet radiation.

4. SOUTHERN TRANSFER / SOUTHERN BLOTTING :-

- The gel is fragile. It is necessary to remove the DNA from the gel and permanently attaches it to a solid support. This is accomplished by the process of Southern blotting. The first step is to denature the DNA in the gel which means that the double-stranded restriction fragments are chemically separated into the single stranded form.
- The DNA then is transferred by the process of blotting to a sheet of nylon. The nylon acts like an ink blotter and "blots" up the separated DNA fragments, the restriction fragments, invisible at this stage are irreversibly attached to the nylon membrane.
- This process is called Southern blot by the name of **Edward Southern (1970)**.

5. HYBRIDIZATION :-

- To detect VNTR locus on restricted fragment, we use single stranded **Radioactive (P^{32}) DNA** probe which have the base pair sequences complimentary to the DNA sequences at the VNTR locus. Commonly we use a combination of at least 4 to 6 separate DNA probes.
- Labelled Probes are attached with the VNTR loci of restricted DNA Fragments, this process is called Hybridization.

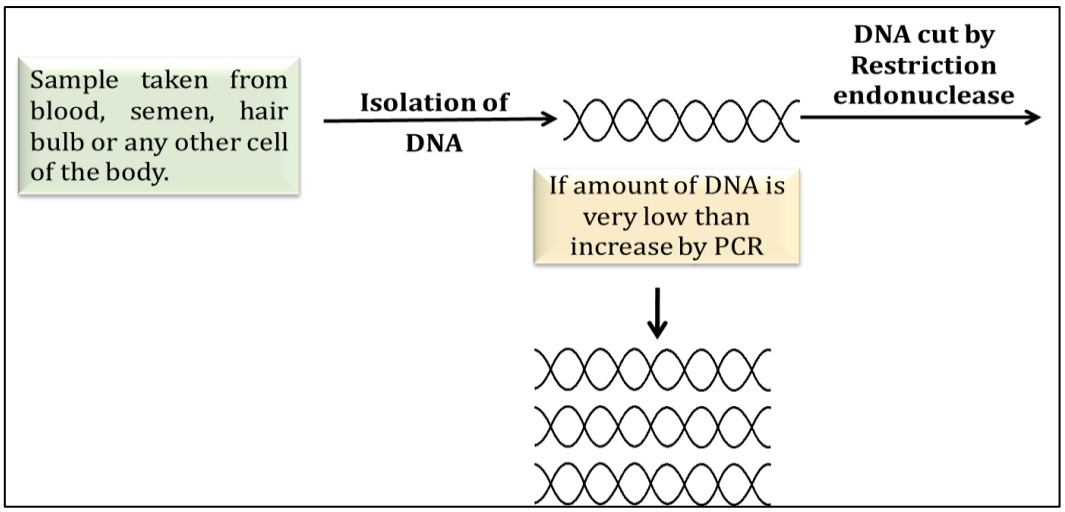
6. AUTORADIOGRAPHY : Nylon membrane containing radioactive probe exposed to X-ray. Specific bands appear on X-ray film. These bands are the areas where the radioactive probe bind with the VNTR.

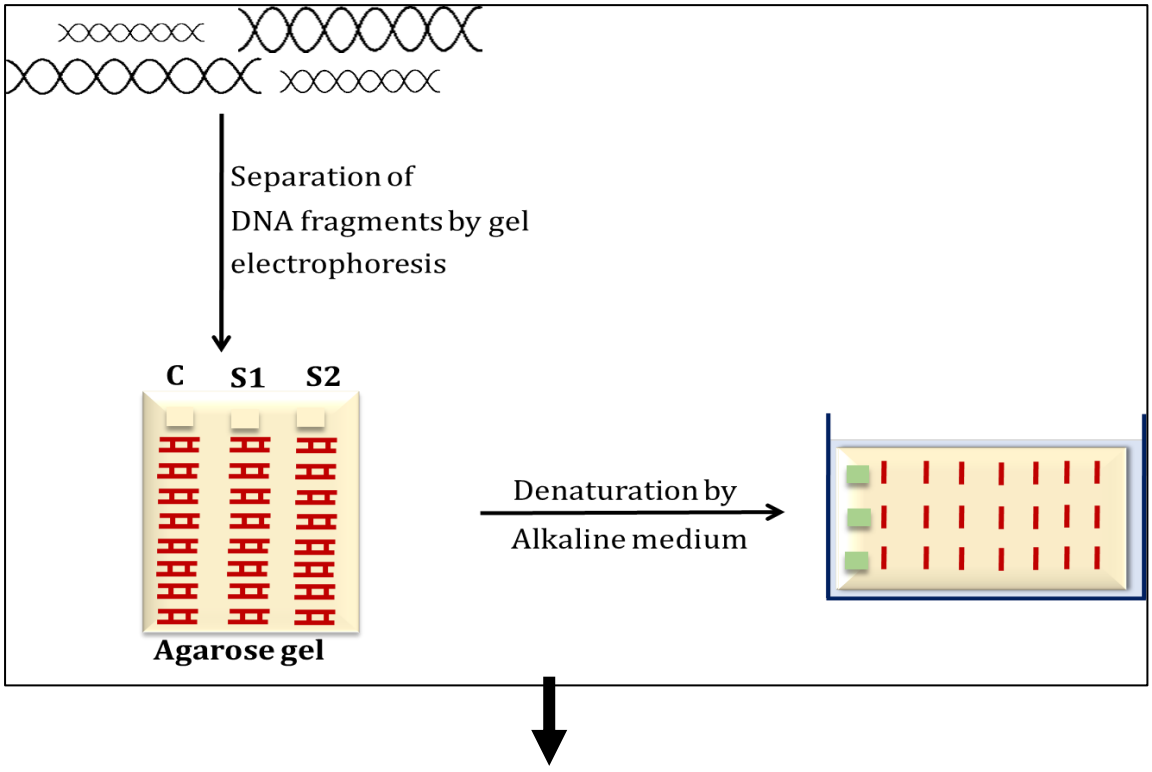
This appears the specific restricted fragment length pattern. This length pattern is different in different individual. This is called Restricted Fragment length Polymorphism (RFLP).

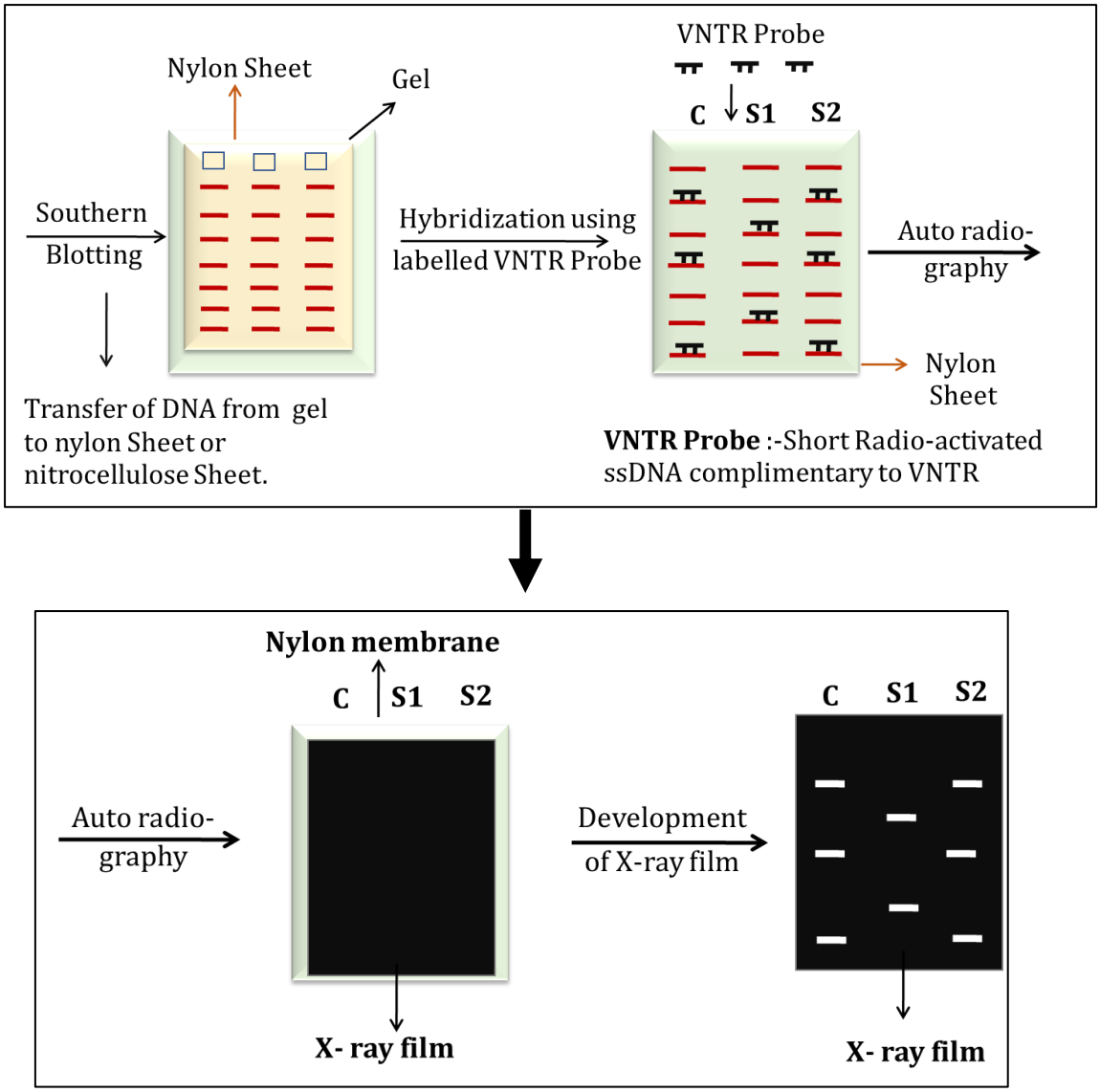
These allow analyzer to identify a particular person DNA, the occurrence and frequency of a particular genetic pattern contained in this X-ray film. These x-ray film called DNA signature of a person which is specific for each individual.

The probability of two unrelated individual having same pattern of location and repeat number of mini satellite (VNTR) is one in ten billion (world population 6.1 billion)

In India the centre for DNA finger printing and diagnosis **(CDFD - Center For DNA Finger Printing & Diagnosis) located at Hyderabad**.


Sample taken from blood, semen, hair bulb or any other cell of the body.
Isolation of DNA
DNA cut by Restriction endonuclease
If amount of DNA is very low than increase by PCR

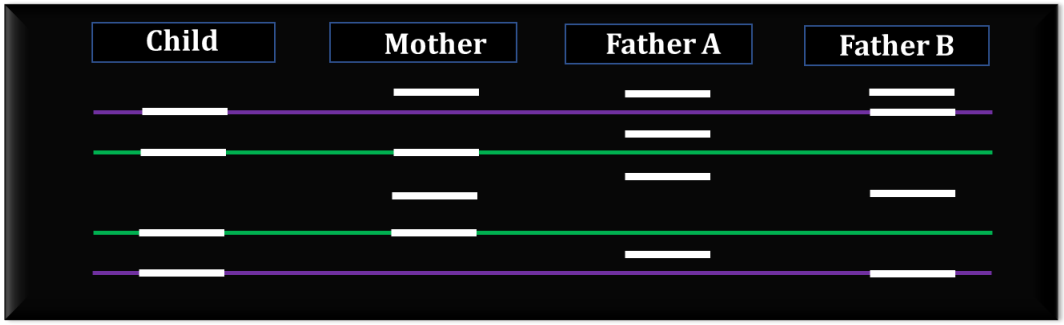

Separation of DNA fragments by gel electrophoresis
C
S1
S2
Agarose gel
Denaturation by Alkaline medium



APPLICATION OF DNA FINGER PRINTING :-

1. PATERNITY TESTS :-

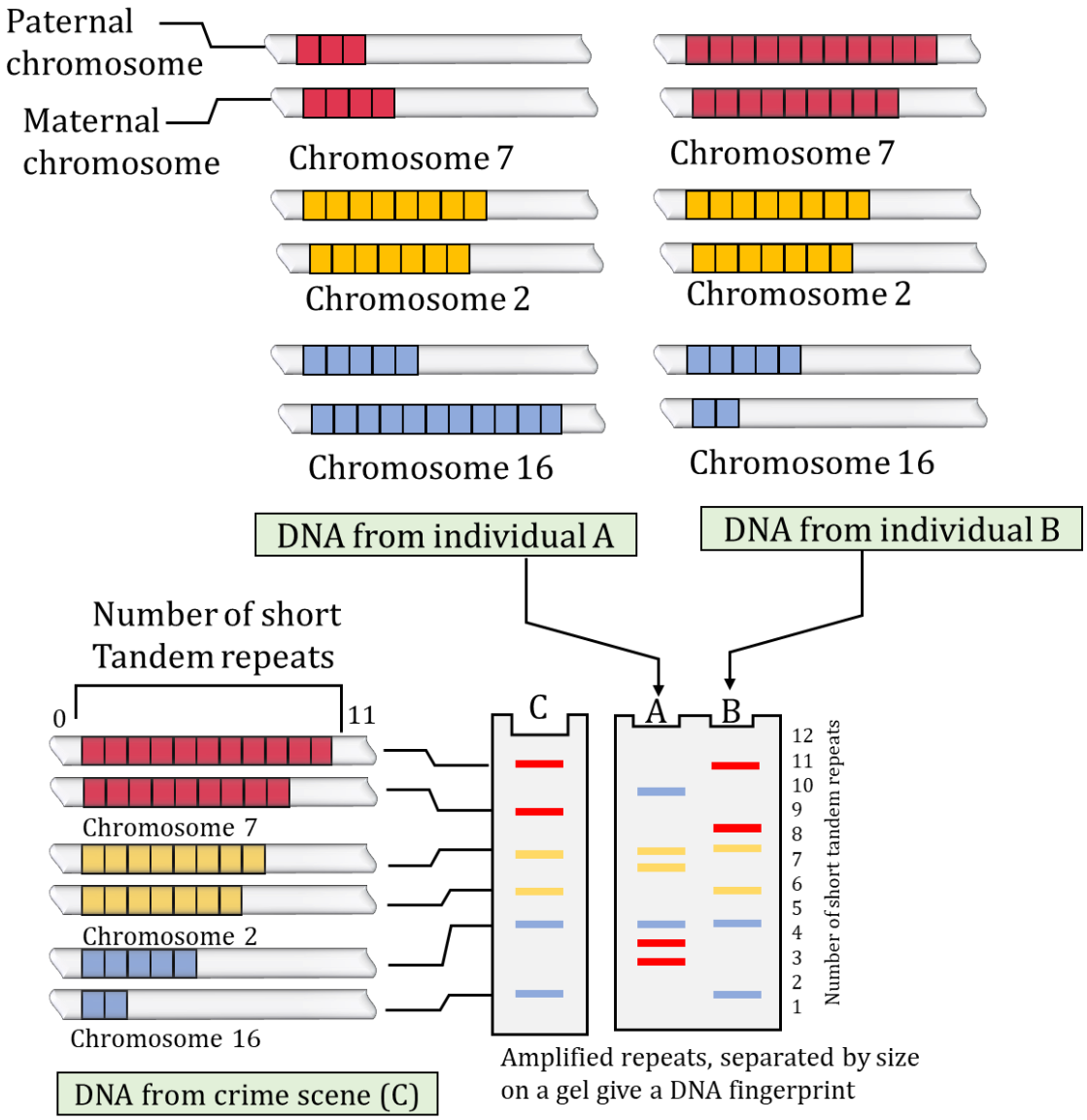
The major application of DNA finger printing is in determining family relationships. For identifying the true (biological) father, DNA samples of Child, mother and possible fathers are taken and their DNA finger prints are obtained. The prints of child DNA match to the prints of biological parents.



In Above case father B is biological father.

2. IDENTIFICATION OF THE CRIMINAL :-

DNA finger printing has now become useful technique in forensic (crime detecting) science, specially when serious crimes such as murders and rapes are involved. For identifying a criminal, the DNA fingerprints of the suspects from blood or hair or semen picked up from the scene of crime are prepared and compared. The DNA fingerprint of the person matching the one obtained from sample collected from scene of crime can give a clue to the actual criminal.



Schematic representation of DNA fingerprinting: Few representative chromosomes have been shown to contain different copy number of VNTR. For the sake of understanding different colour schemes have been used to trace the origin of each contain different colour schemes have been used to trace the origin of each band in the gel. The two alleles (paternal and maternal) of a chromosome also contain different copy number of VNTR. It is clear that the banding pattern of DNA from crime scene matches with individual B. and not with A.

Question-1: DNA finger printing started in India :–

(1) Kary Mullis (2) Dr. K.G. Vaishnav

(3) Dr. Gaurav Maheshwari (4) Dr. Lal Ji Singh and Dr. V.K. Kashyap

Answer: (4) Dr. Lal ji Singh and Dr. V.K. Kashyap

Explanation:

In India DNA Finger printing has been started by Dr. V.K. Kashyap & Dr. Lal Ji Singh.

Question-2: Western blotting is used for the identification of -

(1) DNA (2) RNA (3) Protein (4) All the above

Answer: (3) Protein

Explanation:

Blotting techniques are used to transfer nucleic acids from gels, after gel electrophoresis, to membranes for further analytical studies. Western blotting is used for the identification of protein.

Question-3: Which of the following techniques are used in analysing restriction fragment length polymorphism (RFLP) -

(a) Electrophoresis

(b) Electroporation

(c) Methylation

(d) Restriction digestion

(1) 'a' and 'c' (2) 'c' and 'd' (3) 'a' and 'd' (4) 'b' and 'd'

Answer: (3) 'a' and 'd'